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Randomized controlled trial on a PRP-like cosmetic, biomimetic peptides based, for the treatment of alopecia areata

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ABSTRACT

Background: Alopecia areata (AA) is a non-scarring auto-immune hair disorder. Recent research explained the role of growth factors (GFs) in hair follicle cycling. The main reservoir of GFs are alpha-granules of platelets and novel procedures have been implemented aimed at collecting platelet-rich plasma (PRP). PRP has been safely implemented in many medical applications and has also been successfully used as alternative cell-based therapy for the treatment of hair growth disorders, among which also AA.

Objectives: By means of a randomized double-blinded, placebo and active-controlled, parallel group study we have studied the efficacy of a cosmetic product (named TR-M-PRP plus) comprising biomimetic peptides specific for hair growth, mimicking PRP composition for the treatment of AA. Subjects were treated for three months and evaluated, at the end of the study and after one month of follow-up, as regards hair growth using SALT score.

Results: TR-M-PRP plus-like topic produced a statistically significant ($p<.001$) clinical improvement in SALT score after 3 months of therapy, compared to baseline. Hair growth results further improved after 1 month of follow-up.

Conclusions: This clinical investigation suggests that the biotechnological designed PRP-like cosmetic could represent a valid and safer alternative to autologous PRP for the treatment of AA.

Abbreviations: PRP: Platelet-rich plasma; AGA: Androgenetic alopecia; AA: Alopecia areata; GFs: growth factors; VEGF: vascular endothelial growth factor; EGF: epidermal growth factor; FGF: fibroblast growth factor; IGF: insulin-like growth factor; ADP: Adenosine diphosphate; SALT: Severity of Alopecia Tool; bFGFs: Basic Fibroblast Growth Factor

What’s already known about this topic?

- Platelet-rich plasma (PRP) PRP has been successfully used as alternative cell-based therapies for the treatment of hair growth disorder such as Androgenetic alopecia (AGA) or Alopecia areata (AA).

What does this study add?

- We have studied a topical formulation (named TR-M-PRP plus) comprising biomimetic peptides specific for hair growth mimicking PRP composition.
- The results obtained with the present clinical investigation suggest that the biotechnological designed PRP-like cosmetic we investigated could represent a valid and safer alternative to autologous PRP for the treatment of AA.

Introduction

Alopecia areata (AA), is a non-scarring auto-immune hair disorder [1,2]. Even though AA ethiology is not completely understood, many clinical evidence suggested a role of immunity in the development of such disease [3–5]. Therefore, genetic predisposition, environmental factor, psychological stress, hormonal unbalance, concomitance with other skin disorders and gut dysbiosis can contribute to autoimmune mechanism of AA [1,6,7]. More recently, a role of scalp microbiome has also been hypothesized (8). AA is the second most common type of alopecia with an incidence higher than 2% and a lifetime risk of 1.7% both in men and women (9).

Currently, available treatment options for AA included: topical (10), intra-lesional (11) or systemic (12) steroids, and immunotherapy or systemic immuno-modulators.

Recent researches (13–16) explained the role of growth factors (GFs), especially polypeptide in the life-long cyclic transformation of the hair follicle, and their activity in control of immune privilege (in particular IGF-1) (17). GFs act by stimulating cell proliferation and differentiation and inhibiting apoptosis on dermal papilla cells and stimulate stem cells of bulge area (18–20). This activity will result in anagen prolongation and catagen delaying (21,22).

The main reservoir of growth factors in the body are alpha-granules of platelets (23) and growth factors involved on hair follicle cycling are mainly vascular endothelial growth factor (VEGF) (24) epidermal growth factor (EGF) (25), fibroblast growth factor (26,27) and insulin-like growth factor (IGF) (28). Adenosine diphosphate (ADP), serotonin and calcium are also released from dense granules and are important in the recruitment of new platelets and coagulation cascade (29).
Novel procedures have been implemented aimed at collecting platelet-rich plasma (PRP) (30–34) releasing growth factors after platelets degranulation (29).

PRP derived from autologous blood has a 1,000,000/UL platelets concentration, which is 3–8 folds higher than normal peripheral blood (range 150,000–350,000 UL) (35,36).

First used in 1987 by Ferrari et al. (37) in transfusion procedures, PRP has then been safely implemented in many application fields, such as orthopedics and sports medicine, dentistry, neurosurgery, ophthalmology, urology, and wound healing (38,39). PRP has also been successfully used as alternative cell-based therapies for the treatment of hair growth disorder such as AGA (36,40) or AA (41,42).

In a randomized, double-blind, placebo and active-controlled, a half-head study on AA subjects, Trink and collaborators investigated, for the first time, the safety and efficacy of PRP on hair regrowth and dystrophy, burning or itching (43).

During the time, attempts have been made to standardize procedures for PRP preparation in order to reduce variation in the concentration of platelets and differences in manufacturing procedures (44). With a view to the above limitations, the use of biomimetic peptides, mimicking growth factors normally encountered in PRP, could represent a valid alternative. They can be implemented in an topical formulation and used for the treatment many conditions in which modulation by growth factors is involved.

Normally, a biomimetic peptide is an oligopeptide (10–15 aa) that provides similar efficacy of natural or recombinant growth factors but reduce cost and owns more stability. By mean of biotechnological development, a wide range of biomimetic peptides has been developed since the beginning of 2000. Since their birth, these novel discovered molecules represented a very promising application with regard to skin and dermatological applications (45). Several kinds of biomimetic peptides are currently available on the market. They include signal peptides (46,47), carrier peptides (48,49) and also specific peptides targeting hair such as tripeptide-copper complex (50), 5-aminolevulinic acid-GHK (ALAVAX) (51) Octapeptide-2 (52,53), Decapeptide P3 (54) and S-hexapolyptide 9 (55). In a previous randomized trial on 40 women affected by chronic telogen effluvium, we have evaluated the efficacy of a pool of selected mimicking growth factors (IGF 10%, EGF 10%), included in a topical formulation, in preventing dermal papilla apoptosis, prolong anagen phase and delaying catagen and telogen (56).

More recently we have also evaluated in vitro the efficacy of a mix of biomimetic peptides, the same used in the product covered by the present study, for hair growth stimulation (submitted for publishing). Starting from the previously reported evidence we have developed a cosmetic product (named TR-M-PRP plus) for the treatment of AA, comprising biomimetic peptides specific for hair growth and mimicking PRP composition.

Material and methods

Study design and patients

The study was structured in the form of a randomized double-blinded, placebo and active-controlled, parallel group study. 60 subjects with AA of both sex, aged between 18–60 years, were enrolled. For each AA patient, essential background data were collected at baseline according to the guidelines of the National Alopecia Areata Foundation (57,58). AA-grade was assessed according to Severity of Alopecia Tool (SALT) (57) score (S0 = no hair loss; S1 < 25% hair loss; S2 = 25%–49% hair loss; S3 = 50%–74% hair loss; S4 = 75%–99% hair loss; and S5 = 100% hair loss). Efficacy of TR-M-PRP plus treatment was assessed as percentage hair regrowth and the grading of overall improvement, calculated from change in baseline SALT score. Absolute change in SALT score = SALT score at baseline – SALT score at T2. Percentage of hair regrowth was calculated as follows: 100 × (Baseline SALT score – SALT score at T1 or T2)/Baseline SALT score. Assessment of percentage hair regrowth was graded into following 6 grades: A0 = no change or further loss of hairs; A1 = 1–24% regrowth; A2 = 25–49% regrowth; A3 = 50–74% regrowth; A4 = 75–99% regrowth; AS = 100% regrowth. Subjects had also to accepting to not receive any other drug/cosmetic treatments during the study and had not be involved in a similar study during the previous 6 months. Exclusion criteria included known sensitivity to any compound of the investigational product, pregnancy or breastfeeding, any other medical condition or other scalp or hair disorders.

All patients were evaluated and enrolled in the study by the RS Dermatologic Clinic, Milan, Italy, after signed an informed consent.

The study was under the approval of the Ethical Independent Committee for Clinical, not pharmacological investigation in Genoa (Italy) and in accordance with the ethical standards of the 1964 Declaration of Helsinki.

Table 1. Subjects demographic characteristics.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n, %)</td>
<td>37 (61.67 %)</td>
</tr>
<tr>
<td>Women (n, %)</td>
<td>23 (38.33 %)</td>
</tr>
<tr>
<td>Age (y, mean ± DS)</td>
<td>54.32 ± 8.17</td>
</tr>
</tbody>
</table>

Treatment

AA enrolled subjects were randomly divided into 2 groups: group I included 30 AA patients treated with TR-M-PRP plus; group II included 30 AA patients treated with Placebo. Both groups applied the product twice a week (15 ml) for 3 months. Biomimetic peptides used were: Copper Tripeptide-1, Octapeptide-2, Oligopeptide-20, and Acetyl Decapeptide-3. Lactoferrin, lactoglobulin, and melatonin were also included as an anti-inflammatory, ATP stimulator and circadian rhythm regulator agents, respectively.

Assessment of the response

Subjects have been visited three times: at the Randomization Visit (Baseline T0), at the End of Treatment Period Visit at Month 3 (T1, 90 days), and at the Follow Up Visit, one month after treatment end (T2, 120 days). Photography Digital photos were taken for the scalp before therapy and during subsequent visits. Hair regrowth in AA subjects has been evaluated using the SALT score which expresses hair regrowth as a percentage from baseline (59–61). At the end of the study (T1) and at the Follow up Visit (T2), each volunteer has also filled out a questionnaire regarding the perceived efficacy of the treatment and product compliance.

Statistical analysis

A two-sample Student’s t-test was used for comparison at baseline and during the study. p-values less than .05 were considered clinically significant.

Results

A total of 60 subjects (37 men and 23 women) were enrolled and received treatment (Table1). The TR-M-PRP plus-treated group and
placebo group had comparable baseline demographics and disease characteristics.

Hair-growth measured after 3 months of treatment and a follow-up of one month with TR-M-PRP plus (Group I) were compared with values registered at the baseline and compared to Placebo group (Group II).

Enrolled AA subjects presented a mean of 4.35 symmetrically distributed patches of hair loss and had the last relapse 1–2 years before (mean 1.2). They were no responsive to any other previous treatment including systemic and topical immunosuppressant therapies and phototherapy. Therefore, they received no treatment for at least one year.

Absolute change in the baseline SALT score was calculated. Mean value of the absolute change in SALT score was 18.30 and 8.49 for Group I and II, respectively. Percentage scalp hair regrowth was derived from the absolute change in the baseline SALT score for all the patients. After three months of treatment (T1) the mean values were 57.07% for Group I and 27.96% for Group II (Table 2). At T2 a further significative (p < .0001) improvement was found for Group I (68.12% vs 28.89% in Group II).

In Group I, 53.33% cases showed complete regression (A5) grade) (Table 3). A partial regression was also seen in 13.33% of patients. After six months of treatment (T1) the mean values were 75.09% for Group I and 39.74% for Group II. At T2 a further significative (p < .0001) improvement was found for Group I (86.12% vs 39.74% in Group II).

No adverse effects were reported after TR-M-PRP plus or Placebo administration. Therefore, all patients under investigation reported a good compliance of the tested product.

Explicative photographic images showing hair regrowth effect of TR-M-PRP plus were reported in Figure 1.

### Table 3. Grading of overall improvement in Group I and II.

<table>
<thead>
<tr>
<th>Percentage changes in baseline SALT score</th>
<th>After 3 months of treatment (T1)</th>
<th>After 1 months of follow-up (T2)</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall improvement</td>
<td>Group I</td>
<td>Group II</td>
<td></td>
</tr>
<tr>
<td>A0 (no hair regrowth)</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>A1 (1–24%)</td>
<td>1</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>A2 (25–49%)</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>A3 (50–74%)</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>A4 (75–99%)</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>A5 (100%)</td>
<td>16</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Indeed, the results indicate that the application of biomimetic peptides in patients with AA leads to a decrease in hair loss probably due to prolongation of the anagen phase (probably acting on WNT/β-catenin pathways and via exosomes stimulation) and, consequently, due to the reduction of the telogen phase and possibly, by immunological control.

Following our previous work (43) several published works have been reported as regards efficacy of autologous PRP in AA (64–69). The product under study represents the first biotechnological designed PRP able to reproduce the efficacy of autologous PRP. Many biomimetic peptides are currently available on the market but few reported a well-characterized action on hair growth. We settled up a mix of biomimetic peptides able together to simulate autologous PRP but avoiding its intrinsic limitations (cost, interpersonal variation, invasiveness of the procedure, and reported side effects).

All subjects under investigation well tolerated the treatment and no side effects were identified. Therefore, all used biomimetic peptides are protected by micro-encapsulation to avoid peptidases and proteases degradation and these results in higher stability. Tripeptide-1 (GHK) (Glycyl-l-histidyl-l-lysine) is a biomimetic peptide that is physiologically released during inflammation and wound healing process (48). This peptide shows a high affinity for copper ions, forming a complex (70): Copper Tripeptide-1 (GHK-Cu). It possesses a diverse multiplicity of actions being able to activate many remodeling related processes. Indeed, GHK-Cu is a powerful anti-inflammatory agent in wound-healing (71–74). It also acts on metalloproteinas and on extracellular matrix proteins (70,75–77) and also stimulates angiogenesis (71,78). Most interesting GHK-Cu is able to counteract hair loss through the stimulation of stem cells, increasing hair follicle size (79,80).

We implemented in the formulation also Octapeptide-2 (Thr-Ala-Glu-Glu-His-Glu-Val-Met). It is a mimetic of thymosin-β4 growth factor, a well-known stimulator of hair growth that acts on angiogenesis and promotes the migration of stem cells and their progeny to the base of the follicle. It also stimulates differentiation and extracellular matrix remodeling (53,54).

Acetyl Decapeptide-3 is a Basic Fibroblast Growth Factor (bFGFs) biomimetic whom efficacy has been largely proven in skin regeneration. As biomimetic of bFGFs, it is involved in normal skin growth, healing and wound repair. Most interesting, bFGFs have been shown to be involved in hair development (27,28).

Oligopeptide-20 (BH-Cys-Arge-Lys-Ile-Pro-Asn-Gly-Tyr-Asp-Thr-Leu-OH) is another peptide involved in hair growth mechanisms. It’s supposed to act as an enzyme inhibitor leading to an increase of the synthesis of collagen and glycosaminoglycans. The involvement of collagen, in particular, collagen IV in hair cycling has been recently reported (81). Reduced levels of collagen had also been related to hair follicle aging (82).

We can postulate that the above-reported oligo-peptides may simulate together the efficacy of autologous PRP (43) acting by promoting hair follicle growth probably via stimulation of cell proliferation. Further in vitro experiment could help in confirming mechanism behind their mechanism.

The cosmetic product investigated contained also: (i) lactoferrin that is a potent anti-inflammatory agent (83) for helping in
counteract inflammation mechanisms of AA; (ii) lactoglobulin helpful for stimulating mitosis and ATP production (84); (iii) melatonin for clock gene regulation (85), a process strictly involved in hair growth regulation.

Conclusion

The results obtained in the present clinical investigation suggest that the biotechnological designed PRP-like cosmetic we investigated could represent a valid and safer alternative to autologous PRP for the treatment of AA. Further in vitro and in vivo studies may help into better underline the mechanism behind its efficacy.

Disclosure statement

R.F. and S.E. serve as a consultant for Giuliani S.p.A. P.D. and M.B. are employed by Giuliani S.p.A.

Funding

This study was supported by Giuliani SpA.

References


Figure 1. Explicative photographic images of a subject from Group I before (A) and after (B) the treatment.


83. Zhang K, Letham DS, John PC. Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of p34cdc2-like H1 histone kinase. Planta. 1996;200:2–12.
