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ORIGINAL RESEARCH

Efficacy of Postbiotics in a PRP-Like Cosmetic Product for the Treatment of Alopecia Area Celsi: A Randomized Double-Blinded Parallel-Group Study

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ABSTRACT

Introduction: Alopecia areata (AA), also known as 'area Celsi', is the second most common form of hair loss affecting the scalp. Newly proposed treatments for AA include low-level light therapy, biologics such as Janus kinase inhibitors and autologous platelet-rich plasma (PRP), which is a well-known "elixir" for hair growth. Bioactive peptides developed through biotechnological applications have been used to overcome the limitations of PRP. More recently, the involvement of microbiota in hair growth disorders, in AA in particular, has been reported, and the usefulness of microbial metabolites, i.e. postbiotics, has been suggested.

Methods: This study was a randomized double-blinded parallel-group study in which 160 persons of both sexes affected by AA and aged between 18 and 60 years were enrolled. The subjects were randomly assigned to a treatment group (group 1), receiving the TR-PRP plus-Celsi cosmetic product, and a placebo group (group 2). The SALT (Severity of Alopecia Tool) score was determined in both groups at baseline and after 2 and 3 months of treatment, and the results compared between groups.

Results: The subjects in group 1 showed a significant change from baseline in SALT score at 2 months of treatment (61.04% ± 3.45%; $p < 0.0001$), with a further improvement at the end of treatment (3 months) (69.56% ± 4.32%; $p < 0.0001$). No significant changes from baseline were reported for the subjects in group 2 (T1: 26.45% ± 3.64%; T3: 27.63% ± 7.61%).

Conclusions: The results of this study provide further proof of the efficacy of bioactive peptides that mimick the growth factors present in PRP in subjects affected by AA. They also add to our knowledge of the link between microbiota and hair growth disorders, emphasizing the importance of studies on the microbial community and microbial metabolites as a novel therapeutic approach.

Keywords: Alopecia areata; Bee bread; Biomimetic peptides; Microbiota; Plantaricin A; Platelet-rich plasma; Postbiotics

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Key Summary Points

Why carry out this study?

Alopecia areata (AA), also known as 'area Celsi', is the second most common form of hair loss affecting the scalp. Many new treatments for AA have been developed, including autologous platelet-rich plasma (PRP), well-known as an "elixir" for hair growth, bioactive peptides developed through biotechnological applications to overcome the limitations of PRP and microbial metabolites, known as postbiotics.

The aim of the present study was to investigate the efficacy of a topically applied cosmetic product that mimicks PRP and contains postbiotics for the treatment of AA.

What was learned from the study?

The results provide further proof of the efficacy of bioactive peptides that mimic the growth factors present in PRP in subjects affected by AA.

The results also add to our knowledge of the link between microbiota and hair growth disorders, emphasizing the importance of studies on the microbial community and microbial metabolites as a novel therapeutic approach.

INTRODUCTION

Alopecia areata (AA), also known as 'area Celsi', is the second most common form of hair loss affecting the scalp. AA is an autoimmune disorder characterized by one or more circular bald patches on the head [1]. About 2% of cases spread to the entire scalp (alopecia totalis) or body (alopecia universalis) [2]. An incidence of $> 2\%$ among the general population has been reported, with a lifetime risk of 1.7% for both men and women [3]. AA is mainly related to genetic, autoimmunity and inflammatory factors [4, 5], with the collapse of the immune privilege of the hair follicle reported to play a pivotal role in the pathogenesis of this autoimmune disorder [6].

First-line treatment for AA includes intralesional corticosteroids for mild cases and topical immunotherapy for extensive disease, but medications such as minoxidil, an antihypertensive vasodilator, and bimatoprost, a prostaglandin analogue, are also used, usually in combination with other treatments [7].

Given its typical manifestations and high relapse rates [8], AA is often associated with social and psychological implications [9], and research is ongoing to develop new therapies. Newly proposed treatments include low-level light therapy [10] biologics, such as Janus kinase (JAK) inhibitors [11] and autologous platelet-rich plasma (PRP), which is well-known as an "elixir" for hair growth [12]. The efficacy of PRP has been reported in many clinical studies [13–15], but treatment with PRP has a number of limitations, including the absence of a standardized concentration of platelets, variation in the manufacturing of PRP and some legislative issues. To overcome these limitations, researchers have turned to the use of bioactive peptides obtained through the application of modern biotechnology techniques. These peptides mimic the activities of platelet growth factors, have an efficacy similar to PRP treatment and can be easily included in a topical [16, 17] or, in the near future, injectable formulation. More recently, the involvement of microbiota has been reported in hair growth disorders, in AA in particular [18–20], and the usefulness of microbial metabolites, referred to as postbiotics, has been suggested [21].

In the study reported here, we investigated the efficacy of a topically applied gel formulation that mimics the action of PRP and contains postbiotics for the treatment of AA.

METHODS

A total of 160 male and female subjects who were affected by AA and aged between 18 and

60 years were enrolled in this randomized double-blinded parallel-group study. All patients were evaluated at the RS Dermatologic Clinic, Milan, Italy after written informed consent had been given. The study was approved by the Ethical Independent Committee for Clinical, not pharmacological investigation in Genoa (Italy) in June 2016 under the reference number Rif. 2019/06 and all procedures were in accordance with the ethical standards of the 1964 Declaration of Helsinki, as revised in 2013 concerning human rights. All patients provided written informed consent prior to inclusion in the study. The inclusion and exclusion criteria are given in Table 1.

Differential diagnoses of acute telogen effluvium, androgenetic alopecia and cicatricial alopecia in a pattern distribution were considered for all enrolled subjects [22]. Consequently, the affected area of the scalp of all enrolled subjects was also examined by polarized light dermoscopy at ×100 magnification (Molemax HD; Derma Instruments, Vienna, Austria), and the Molemax software tool integrated with the system was used for acquiring the images. A representative image is shown in Fig. 1. Dermatoscopic examination of all patients confirmed the presence of yellow dots and dystrophic hairs, as well as of cadaveric (black dots) hairs, all of which are manifestations typical of AA and occur in 95% of patients at all stages of the disease [23, 24]. These findings were, in some cases, corroborated by histopathological analysis.

Enrolled subjects were randomly assigned to the group receiving the TR-PRP plus-Celsi cosmetic product (group 1) or the placebo group (group 2). All subjects in both groups topically applied about 2 mL of the product/placebo per day (to be applied for least 5 h) for 3 months. The TR-PRP plus-Celsi product was prepared in the form of a semisolid non-ionic gel that contained all of the active ingredients and all of the excipients needed for stabilization and preservation. The main active ingredients of the TR-PRP plus-Celsi gel are biomimetic peptides (copper tripeptide-1, octapeptide-2, oligopeptide-20, acetyl decapeptide-3), postbiotics (plantaricin A [Pln A] and *Lactobacillus kunkei* bee bread, a fermented product and postbiotic

Table 1 Inclusion/exclusion criteria

Inclusion criteria	Exclusion criteria
Male or female aged 18–60 years	Known sensitivity to any compound in the Investigational product
Suffering from AA for at least 3 years	Women who were pregnant or breast feeding or who were planning a pregnancy
AA with a SALT score between S2 and S5	Serious intercurrent infection or other active disease up to 3 months prior to study entry.
Condition not responsive to other previous treatments, either systemic, topical or phototherapy	History of concurrent malignancy
Subjects agreeing to follow the instructions received by the investigator and able to return to the study center at the established times	History of significant alcohol or drug abuse
Subjects agreeing to not use any drug/cosmetic treatment able to interfere with the study results	Significant psychosocial or psychiatric disorders that may impair the subject's ability to meet the study requirements
No participation in a similar study at the present time or during the previous 6 months	Significant concurrent medical disorders that may impair the subject's ability to participate over the whole 1 year of the study
Not pregnant or breastfeeding	Any other medical condition which the Investigator believed would prevent the participant from taking part in the study

Table 1 continued

Inclusion criteria	Exclusion criteria
Subjects agreeing to sign the informed consent form	

AA Alopecia areata, *SALT* Severity of Alopecia Tool

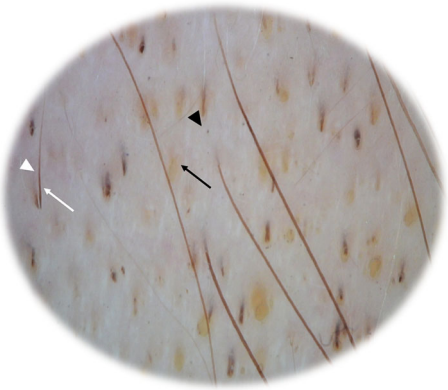

Fig. 1 Representative dermoscopic image from enrolled subjects at baseline showing yellow dots (black arrow), black dots (black triangle), dystrophic hairs (white arrow) and exclamation mark (white triangle)

Table 2 The Severity of Alopecia Tool score

SALT score	Description
S0	No hair loss
S1	< 25% hair loss
S2	25–49% hair loss
S3	50–74% hair loss
S4	75–99% hair loss
S5	100% hair loss

[bee bread]) and *Tropaeolum majus* flower/leaf/stem extract (which provides a gust of oxygen).

Subjects visited the clinic on three different occasions: at the randomization visit (baseline [T0]), after 2 months of treatment (T1; 60 days) and at the end of the treatment period at month 3 (T2; 90 days). The Severity of Alopecia Tool (SALT) score was used to assess the efficacy of the treatment. Digital photographs were taken at each visit. The AA SALT score was assessed according to the guidelines of the National Alopecia Areata Foundation (Table 2) [25], with S0 indicating no hair loss; S1, < 25% hair loss; S2, 25–49% hair loss; S3, 50–74% hair loss; S4, 75–99% hair loss; S5, 100% hair loss.

The efficacy of the treatment was assessed as the change in SALT score relative to baseline, as follows: 100 × (baseline SALT score − SALT score at T1 or T2)/baseline SALT score. The percentage of hair regrowth was also calculated according to a 6-level scale, with A0 indicating no change or further loss of hairs; A1, 1–24% regrowth; A2, 25–49% regrowth; A3, 50–74% regrowth; A4, 75–99% regrowth; A5, 100% regrowth.

At the end of the study (T2), each subject completed a questionnaire on the perceived efficacy of the treatment and product compliance.

Data were collected, and a two-sample Student's *t* test was used for statistical analysis. *P* values of < 0.05 were considered to indicate clinical significance.

RESULTS

A total of 160 persons (Table 3) suffering from AA (SALT score S2–S5) were enrolled in the study and randomly assigned to the TR-PRP plus-Celsi group (group 1) or the placebo group

Table 3 Baseline demographic characteristics of the subjects randomized to the two study groups

Demographic characteristics	Group 1 (N = 80)[a]	Group 2 (N = 80)[a]
Men	44.00 (55.00%)	37 (47.50%)
Women	36.00 (45.00%)	43.00 (52.50%)
Age (years)	51.84 ± 9.54	53.12 ± 6.18
Number of patches	3.54 ± 1.63	3.79 ± 2.01

Values in table are presented as a number with the percentage in parenthesis or as the mean ± standard deviation (SD)

[a] Group 1 subjects received the TR-PRP plus-Celsi cosmetic product as treatment; group 2 subjects received placebo

(group 2). The subjects in the two randomized groups were found to have comparable demographic characteristics.

All of the enrolled subjects had not been responsive to earlier treatments, either systemic, topical or phototherapy. The last treatment had been at least 1 year earlier.

The percentage of hair loss relative to baseline was calculated as the sum of five scalp areas (vertex, right profile, left profile, posterior aspect of scalp). The results of these calculations relative to baseline (T0) at 2 (T1) and 3 months (T2) of treatment are reported in Table 4. The subjects in group 1 showed a significant ($p < 0.0001$) change from the baseline SALT score at 2 months of treatment (61.04% ± 3.45%), with a further improvement observed at the end of the treatment (T2, 3 months) (69.56% ± 4.32%; $p < 0.0001$). No significant changes from baseline to T1 and T2 were observed for the subjects in group 2 (T1: 26.45% ± 3.64%; T2: 27.63% ± 7.61%).

Grading of the overall improvement in the subjects in group 1 and 2, respectively, is reported in Table 5. In group 1, 47.50% of subjects achieved a complete regression (A5) and, in line with results from previous studies [16], 13.75% achieved a partial regression (A3); only 6.25% of subjects in group 1 reported no response at all (Table 5). In contrast, only 5% of the subjects in group 2 reported a complete regression (Table 5).

No adverse effects were reported from the subjects in both groups, and all subjects were in good compliance with the tested products.

An explicative dermoscopic image after treatment is shown in Fig. 2. Figure 3 shows explicative photographic images of the effect of the TR-PRP plus-Celsi treatment on hair regrowth in three subjects in group I.

Table 5 Grading of overall improvement in group I and group II subjects

Overall improvement	Group 1 (*N*)	Group 2 (*N*)
A0 (no hair regrowth)	5	29
A1 (1–24%)	6	34
A2 (25–49%)	5	8
A3 (50–74%)	11	5
A4 (75–99%)	15	0
A5 (100%)	38	4

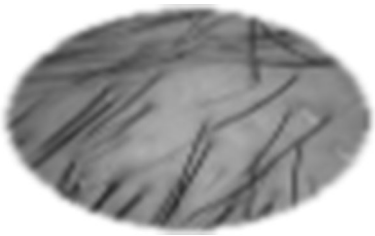

Fig. 2 Representative dermoscopic image from enrolled subjects at baseline (T0)

DISCUSSION

Various treatment options are available to clinicians for the management of hair growth

Table 4 Percentage changes in baseline Severity of Alopecia Tool score in group 1 and group 2 subjects

Percentage changes in baseline SALT score	After 2 months of treatment (T1)	After 3 months of treatment (T2)	Statistical analysis (two-sample Student's *t* test)[a]		
			T0 vs. T1	T0 vs. T2	T1 vs. T2
Group I	61.04 ± 3.45	69.56 ± 4.32	< 0.0001	< 0.0001	< 0.0001
Group II	26.45 ± 3.64	27.63 ± 7.61	0.040	0.715	0.956

Values in table are presented as the mean ± SD

[a] T0, Baseline; T1, after 2 months (60 days) of treatment; T2, after 90 days (end of treatment period)

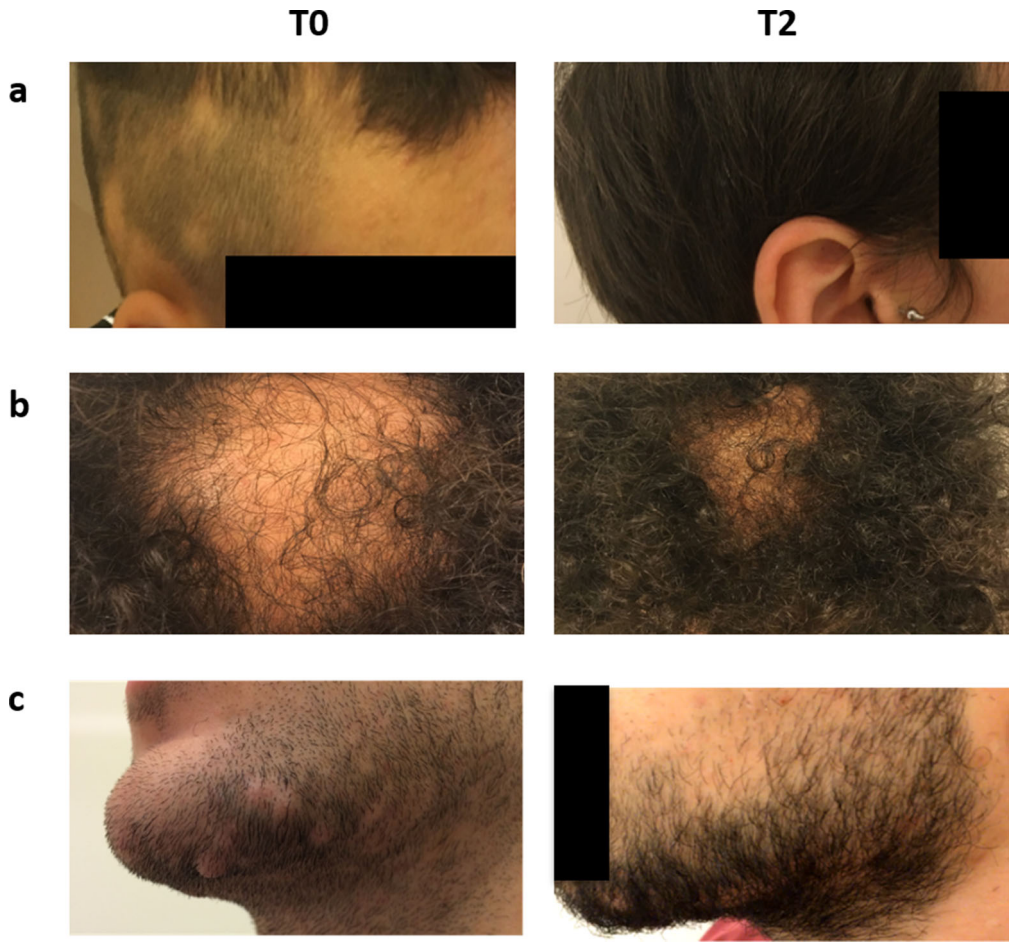

Fig. 3 Digital photographs from three different subjects (**a**, **b**, **c**). Baseline (T0), 3 months of treatment (T2)

disorders (AA, androgenetic alopecia [AGA], lichen planopilaris) [7, 26, 27] Autologous PRP is a relatively new therapeutic approach for patients suffering from alopecias that has achieved a high degree of compliance among patients [15, 28, 29]. It differs from other treatments in terms of its mechanism of action, which is based on the actions of the growth factors contained in the formulation, such as platelet-derived growth factor, vascular endothelial growth factor and transforming growth factor, among others, in regulating cell migration, proliferation, remodeling of the extracellular matrix and promotion of endothelial permeability [30].

Platelet growth factors can interact with both dermal papilla cells and cells from the bulge area, with the effect of activating the proliferative phase of the hair [31–35].

A large number of biomimetic peptides have been developed to overcome the limitations of autologous PRP [36]. Short chains of 10–15 amino acids act similarly to natural growth factors by mimicking their structure or activity, or both [37]. Such peptides possess a higher stability and specificity, are less expensive than PRP and can be easily included in a topical formulation. These benefits have been shown in many applications, ranging from skin rejuvenation [37, 38] to wound-healing [38] and hair growth [39–44].

In our study, we reported the efficacy of a PRP-like cosmetic gel containing postbiotics for the treatment of AA. Of the 160 subjects enrolled in the study, a significant

improvement in hair regrowth was recorded in the majority of the 80 subjects treated with the active product. These results are in line with those from our previous studies in which we demonstrated the efficacy of a pool of peptides derived by biotechnology that mimic platelet growth factors in AA [16] and AGA [17]. These peptides include copper tripeptide-1, oligopeptide-20, acetyl decapeptide-3 and octapeptide-2. Copper tripeptide-1 (copper-glycyl-L-histidyl-L-lysine [GHKCu]) [45] stimulates stem cells in the hair follicle [46, 47], possesses a remodelling activity [48–51] and stimulates metalloproteinases [45, 52–54] and angiogenesis [48, 55–57]. Oligopeptide-20 (8H-Cys-Arg-Lys-Ile-Pro-Asn-Gly-Tyr-Asp-Thr-Leu-OH) is involved in hair growth mechanisms by increasing the synthesis of collagen and glycosaminoglycans [58], thereby preserving hair follicles from aging [59]. Acetyl decapeptide-3 acts as a biomimetic of basic fibroblast growth factor and has been reported to stimulate hair growth [60, 61]. Octapeptide-2 (Thr-Ala-Glu-Glu-His-Glu-Val-Met) is a biomimetic of the hair growth stimulator thymosin-b4 [40, 41]; most interestingly, it also possesses a strong antimicrobial activity [62].

Two other main active components of the TR-PRP plus-Celsi cosmetic product are Pln A and *Lactobacillus kunkeei*-fermented bee bread (bee bread); both can be considered to be "postbiotics". Postbiotics are class molecules with health-promoting effects that are derived from microorganisms, usually probiotics [63]. More specifically, they are substances (metabolites, enzymes, bioactive peptides, short-chain fatty acids, antimicrobial peptides, polysaccharides, cell surface proteins, vitamins, plasmalogens and organic acids) produced by beneficial bacteria as a metabolic product [64] that possess several functional properties, mainly antimicrobial, antioxidant and immunomodulatory in nature [63]. It has been hypothesized that these substances are responsible for probiotic efficacy. Therefore, compared to probiotics, postbiotics can interact easier with both the microbiome and human cells of the host [65]. Other advantages derived from the use of postbiotics are (1) no need for survival or propagation; (2) greater absorption, distribution and extraction potential than probiotics; (3) a safer profile [66].

Pln A and bee bread are obtained by fermenting a raw matrix with lactic acid bacteria. PlnA is an antimicrobial peptide with a pheromone-like activity, produced as the result of quorum sensing between two lactic acid bacteria [67]. It possesses proliferative and wound-healing activities and is also able to induce key mediators of the proliferation, migration and differentiation of epithelial cells [67, 68]. Bee bread is the fermented endproduct of bee-collected pollen [69, 70]. In vitro tests on human keratinocytes (patent bee bread) have demonstrated the immunomodulatory effect of bee bread. Both of these postbiotics have a strong antioxidant activity [68–70]. Regarding the endproducts of microbial metabolism, postbiotics can also be positively sensed by the resident microbiota of the host, modulating the network pathways of both the microbiota and the host.

In an earlier study on microbial dysbiosis on the scalp of AA subjects, we hypothesized a correlation between the presence of some microbial strains in the perifollicular region of AA subjects and hypoxia [18]. This hypothesis has led to novel therapeutic approaches aimed at resolving microbial dysbiosis, including via oxygenation. In this context, the TR-PRP plus-Celsi cosmetic product also contains *Tropaeolum majus* flower/leaf/stem extract, which is a natural active ingredient derived from the nasturtium flower that boosts oxygenation by significantly increasing the activity of hypoxia-inducible factor 1-alpha [71].

CONCLUSION

The results of this study provide further proof of the efficacy of bioactive peptides that mimic the growth factors present in PRP in subjects affected by AA. They also add to our knowledge of the link between microbiota and hair growth disorders, emphasizing the importance of studies on the microbial community and microbial metabolites as a novel therapeutic approach.

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Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility 3for the integrity of the work as a whole, and have given final approval for the version to be published.

Disclosures. Fabio Rinaldi and Anna Trink serve as a consultant for Giuliani S.p.A. Daniela Pinto is employed by Giuliani S.p.A.

Compliance with Ethics Guidelines. The study was approved by the Ethical Independent Committee for Clinical, not pharmacological investigation in Genoa (Italy) in June 2016 under reference number Rif. 2019/06, and all procedures were in accordance with the ethical standards of the 1964 Declaration of Helsinki, as revised in 2013, concerning human rights. All patients provided written informed consent prior to inclusion in the study.

Data Availability. The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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