Title

Impact of L-arginine on androgenetic alopecia: Current clinical data evaluation.

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Abstract

Androgenetic alopecia (AGA) is the most common form of hair loss in men. The phenotype is characterized by a progressive loss of hair from the scalp. It is known that androgens and genetic factors play an important role in onset and may commence during puberty.
Various approaches to treat this condition or at least to attenuate the process are available. Only a few of them have demonstrated efficacy and tolerability by clinical evaluations.
Aim of this report is to summarize data of clinical testing of L-arginine containing treatment options with respect to mode of action, efficacy and tolerability.
Data discussed support the hypothesis of L-arginine absorption by the skin and hair follicles implying a potential modulatory impact on the nitric oxide (NO) pathway. Further data from different observational studies revealed that a topical applied L-arginine solution has a positive impact on reducing the progression of hair loss without any side effects.

Keywords: alopecia, l-arginine, NO, hair loss

Introduction

Androgenetic alopecia (AGA) is a very common non-scarring disease that affects both, men and women [1]. For Caucasian male, it is described that up to 50% of the population is affected by middle age [2]. Genetic and hormonal (androgens) factors are described to be involved in the pathogenesis of androgenetic alopecia. [3] Typically a gradual conversion of terminal hairs into indeterminate, and finally into vellus hairs is observed. Androgen stimulation of the hair follicles result in miniaturization of such and replacement of large pigmented terminal hairs with shorter, thinner, depigmented vellus hairs. Apart from this genetically determined parameter, various environmental factors like nutrition, smoking or UV radiation influence the pathogenesis of AGA [4, 5]. In addition, recent studies suggest that inflammatory processes in the hair follicles may have a pathogenic impact in AGA. [6]. Inflammation and oxidative stress are closely linked in biological systems [7, 8] so it is supposed that dermal papilla cells of patients with androgenetic alopecia are experiencing increased oxidative stress [9].

Although AGA is not a life-threatening condition, it often has a significant impact on the outward appearance of the patients with sometimes-severe social implications particularly in younger men [1]. Due to the constant progression of AGA, a treatment should be started as early as possible and continued indefinitely, since the benefit will not be maintained when the therapy is abandoned.

As many pathogenic mechanisms considered to be involved in the onset of AGA, various treatment options are available. Topical and systemic acting drugs or ingredients, individually or combined, may be used and the choice depends on various factors including grading of AGA, efficacy, practicability, costs and risks [10].

There is a large number of preparations available on the cosmetics market claiming to promote hair growth. Nevertheless, the actual therapeutic mechanism of action is known in only a minority of cases.

One approach addressed recently to interfere with the progression of hair loss in AGA is the topical application of L-arginine. L-Arginine is a semi-essential amino acid and the substrate of the enzyme nitric oxide synthase (NOS), which is responsible for the production of nitrogen monoxide (NO) [11]. There are meanwhile a number of promising findings from studies involving the intravenous or oral administration of L-Arginine in humans, which reflect a wide range of doses, study durations and surrogate parameters for the endothelial function. For example, it has been demonstrated that administering of L-Arginine can improve the endothelial function in patients with hypercholesterolemia [12] and hypertension [13].

Wolf et al suggested that NO acts as a signalling molecule in human dermal papilla cells and implicate basal and androgen-mediated NO production to be involved in the regulation of hair follicle activity [14], which led to the hypothesis that l-arginine might be an interesting

modulator of hair growth. To investigate this hypothesis further, a first step was to determine whether the arginine and asymmetric dimethylarginine (ADMA) content or the relative proportions of the two substances in hair of patients are influenced by the application of an L-arginine containing hair tonic. Both molecules are discussed to influence the NO-Synthesis as source (arginine) or inhibitor (ADMA) [15] [16] [17]

In this article, we present and discuss data from clinical studies exploring the basic hypothesis (molecular mode of action) how L-arginine may influence hair loss as well as clinical outcomes from observational studies of persons with androgenetic hair loss treated with a L-arginine containing hair tonic water for several months.

Methods

L-arginine and ADMA content determination in hair

The study was performed at the Institute of Clinical Pharmacology in Magdeburg.
According to the study design arginine and ADMA (asymmetric dimethylarginine) content in the hair of human volunteers before and after a three weeks' period of treatment with a l-arginine containing hair tonic was measured. The study was performed including forty subjects (20 male and 20 female) as a randomised, placebo-controlled study. 20 subjects received a hair tonic with L-Arginine and 20 received the hair tonic without L-Arginine. No exclusion criteria were defined. Information as to the subjects' age, size, weight, hair status (whether or not dyed), and frequency of hair wash were all recorded prior to the start of the study. The tonic was applied to the scalp twice a day over a period of three weeks, and massaged into the skin thoroughly for 1-2 minutes after every application.
The quantitative analysis of arginine and ADMA was performed according to the method described by Martens-Lobenhoeffer et al.[18] based on high-performance liquid chromotography – tandem mass spectrometry (LC-MS/MS).This procedure is able to determine precise quantitative results for arginine and ADMA, free from interference from other endogenous substances, from numerous biological sources. To determine the content of both amino acids in hair, the calibration range of the procedures for arginine (1,600-16,000 µmol/l) and ADMA (1-10 µmol/l) was modified and the sample preparation procedure adapted. Thereafter the procedure was revalidated to ensure accurate data.

Sample taking and measurement:

Before starting the treatment and after 3 weeks' treatment period approximately 10mg of hair was removed from each subject. In order to ensure comparable results, hair was primarily taken from the subjects' exterior occipital protuberance. If this was not possible, the position

from which the hair was removed was recorded. The hair was bound prior to cutting, with a thread positioned 2-3cm from the scalp. The bound hair was cut directly at the scalp. Each of the removed hair samples was rolled into a piece of aluminium foil and attached to a piece of notepaper using adhesive tape.

To prepare the samples, 8-12mg of hair per test subject and date was weighed and the precise mass of each hair sample recorded. These samples were placed for reaction in sealed polypropylene tubes for 16 hours in 1ml of 6M HCl at 100°C, so as to break down the protein structure and release the amino acids, complete with the arginine and ADMA. In the validation phase of the procedure, it was established that both free arginine and free ADMA were able to overcome the reaction conditions without degradation. After cooling, 10µl was taken from reaction mixtures obtained in accordance with the conditions described above, brought to pH 4.5 with a buffer solution. Isotope-labelled internal standards ($^{13}C_6$-arginine and $^{2}D_7$-ADMA) and acetonitrile were added, to prepare the samples for injection into the LC-MS/MS device.

The direct test results, which were available as µmol/l, were divided by the mass of the hair samples in the reaction mixtures and multiplied by the molar mass of arginine / ADMA, to obtain the final results, in the unit mg/g of hair: These were the figures used in the statistical evaluation. The relative standard deviations of the test results were established by repeated determination for identical hair samples; these were ±4.56% for arginine and ±6.75% for ADMA.

Statistical evaluation

All 40 subjects included in the study completed the study and all samples taken were analysed. In the statistical analysis, which constituted repeat measurements (inter-subject factor: time [basal; 21 days treatment]; inter-subject factor. treatment [placebo, verum [19]] and inter-subject factor sex [female, male]), a significant treatment/time interaction resulted with respect to the concentration of arginine per gram of hair (F=11.4; p=0.002). This can be attributed to the significant increase in arginine concentration in the hair, found only in the group treated with arginine. In the placebo group, the arginine concentration in the hair remained statistically constant, whereby for the men in the placebo group, a trend towards falling arginine concentration was noted (time/sex interaction F= 2.96; p= 0.094).

Observational studies

In total 3 observational studies were performed at Dermatest GmbH (Münster) and Tricholog GmbH (Freiburg). Prior to inclusion in the studies, all subjects signed a written informed consent. Objective of all studies was to determine the influence of an L-arginine containing

hair tonic on androgenetic alopecia. The evaluations were designed as prospective, unblinded and uncontrolled observational studies.

The L-arginine containing tonic was provided by MyDok. The tonic contained Ethanol, Propylenglycol, L-Arginine and water (demineralised).

The participants applied the MY DOK tonic twice a day (morning and evening, massage area where tonic was applied for 3-5 minutes) according to a dermatological protocol.

The three studies described had different treatment periods of 4,6 and 12 months respectively.

At the beginning of each study, a careful examination of the subjects was performed regarding clinical status, dermatological status, and medical history.
Following parameter were analysed:

- Hair density
- Hair volume
- Tolerability

Hair number and denseness was determined by microscopy (TrichoScan method [20]) and photos taken from the analysed area.
An extensive questionnaire was given to the subjects to complement the dermatological evaluation by the experiences of the subjects using the hair tonic.

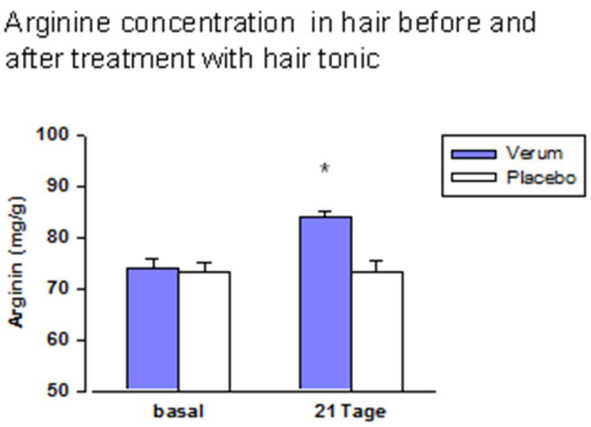
Follow-up examination was performed every month with a photographic documentation. TrichoScan examinations were done at the beginning and end of the treatment of test persons in each study.

Results

L-Arginine and ADMA content measurements

All test subjects completed the study. No side effects were reported, apart from a "certain stickiness" of the hair after application of the tonic, for which reason several test subjects washed their hair more frequently than usual. Examination following de-blinding showed that this effect was independent of the arginine content of the tonic, and that it was therefore attributable to its basic composition.

Figure 1: A) Arginine content in the hair of placebo group and verum group as average concentration of all 20 subjects



B) Arginine content in the hair of placebo group and verum group as average concentration of all female and male subgroups

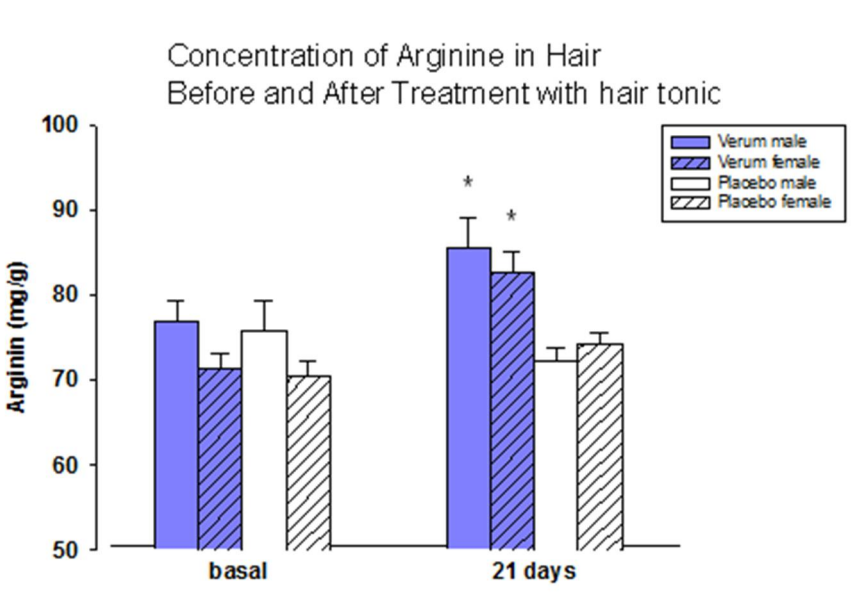
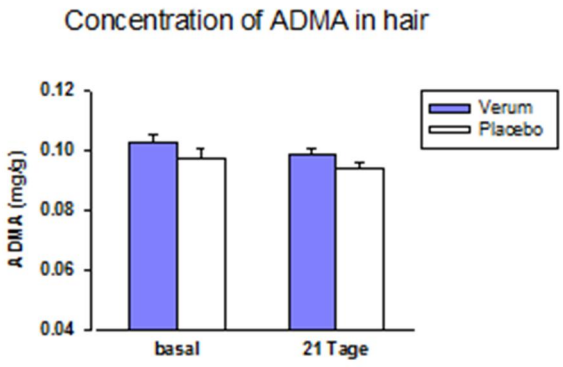
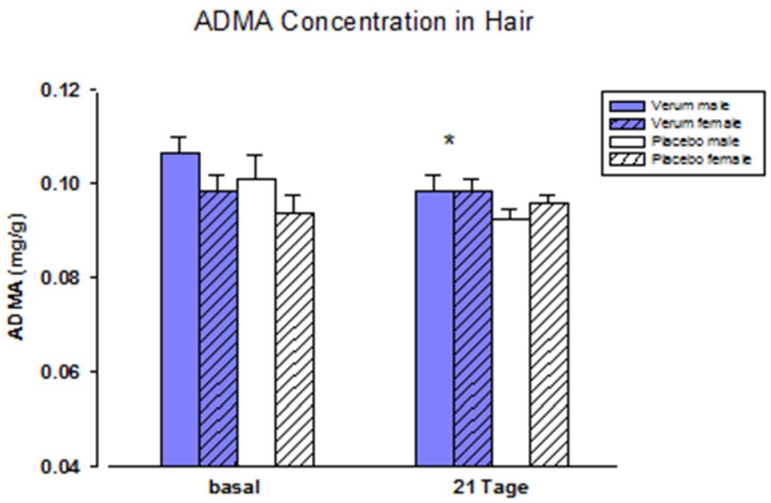


Figure 2: A) ADMA content in the hair of placebo group and verum group as average concentration of all 20 subjects; B) Arginine content in the hair of placebo group and verum group as average concentration of all female and male subgroups

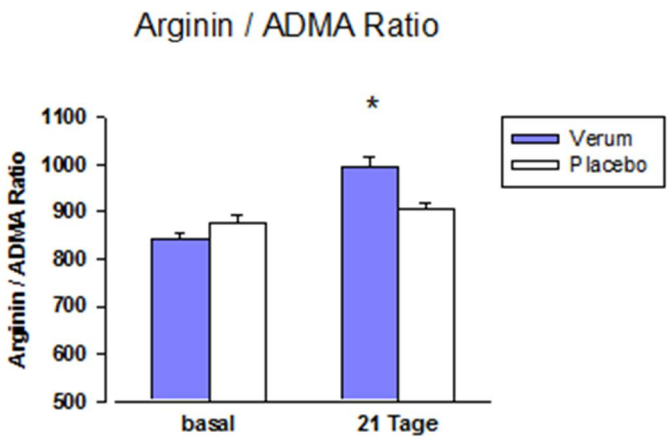


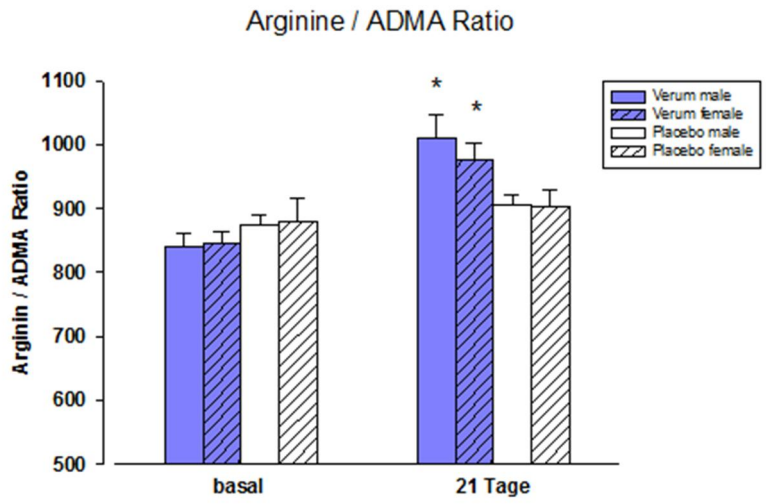


The endogenous inhibitor of the NO synthase, ADMA, remained statistically constant. In this case, there was a significant time/sex interaction (F=5.89; p=0.02), which is attributed to the fact that the tonic treatment among men leads to a significant reduction in ADMA concentration with or without arginine, while this is not the case with women.

The most significant parameter, at least from a therapeutic point of view, is the Arginine/ADMA ratio, which describes the relationship of substrate availability and inhibitor (ADMA) to the NO synthase. This revealed an extremely large treatment/time interaction (F= 26.6; p<0.0001). Among men, a 20% increase in the arginine/ADMA ratio was attained, while the level among women was 15%. This difference is, however, not statistically significant, which means that men and women are able to benefit to the same extent.

Figure 3: A) Arginine / ADMA ratio in the hair of placebo group and verum group as average concentration of all 20 subjects; B) Arginine / ADMA ratio in the hair of placebo group and verum group as average concentration of all female and male subgroups





Observational studies

Three studies with six, twelve and thirty month's observation time were performed to evaluate the beneficial effect of L-arginine containing hair tonic on androgenetic alopecia. In total 127 subjects were included in the three studies (age range between 16-74 years). The studies were dermatologically controlled and revealed that the L-arginine containing hair tonic is well tolerated without any pathological changes or reaction of the skin.

Study 1: Six months follow-up study (Study duration 6 months, Tricholog GmbH (Freiburg)*)*
In a six months follow-up study, 26 female subjects with progressing alopecia were included. At three time points (visit 1= day 0, visit 2 = 3 months, visit 3 = 6 months) hair density, hair strength and type of hair (Vellus haisr/terminal hair) was analysed at a defined test area areal on the scalp. 26 subjects were analysed at Visit 1, 19 at Visit 2 and 13 at Visit 3. A comparison of the data of hair density and strength between the time points V1-V2 and V1-V3 showed for both, 3 months and 6 months treatment period, no statistical significant differences. These results suggest a stop of the hair loss or at least a slowdown of the process by the applied treatment regimens. Furthermore, no adverse effect was reported by any of the re-appearing subjects.

Study 2: Dermatological controlled observational study of test persons with androgenetic alopecia. (Study duration 12 months, Dermatest GmbH (Münster)*)*

In this study, 16 male and 3 female persons with androgenetic alopecia were treated for 12 months with the L-arginine containing hair tonic as described above. All participants tolerated the tonic solution very well without any skin irritation or allergic skin reaction.
9 of 19 subjects (47%) were classified as responder and showed an average increase in hair quantity of 27.8% and 25% in hair density. In addition, the rate of Anagen hair (growth phase) and Telogen hair (resting phase) was determined. Over the 12 months treatment period in the responder group a decrease of Anagen hairs by 12% (72,9% to 64%) was detected while the number of Telogen hairs increased by 9% from 27.1% to 36.1%.
In contrast, a decrease of hair quantity (-24.6%) and hair density (-19%) was measured for 10 probands classifying them as non-responder. Interestingly, in this group the quantity of active hair (Anagen hair) was reduced only by 4% while the number of resting hair increased 12% which may indicate a positive effect on alopecia androgentica too.

Study 3: Dermatological controlled observational study oftest persons with androgenetic alopecia with at least four months treatment (Study duration 30 months, Dermatest GmbH (Münster)*).*

82 test persons were included (age between 16-74 years) in this study and treated for at least 4 months continuously. All subjects included in the study were tested once a month regarding hair loss, hair growth and hair quality and other dermatological relevant aspects. In addition a photographic documentation was performed.

40 test persons patients (48,7%) showed an increase in hair growth (16 cases with definite increase and 24 cases with slight increase). They reported that the hair growth was induced after several years of baldness. Hair loss was ceased in 32 persons (39%) after 4 months treatment. Hair density and hair volume improved in eight cases dramatically while in 24 cases a slight improvement was observed.
From 16 subjects showing scaling and scurf at the beginning of the study, only 1 person remained with mild scurf and scaling after 4 months treatment with the tonic. Furthermore, 3 persons with a vigorous seborrheic scalp eczema revealed to be free of symptoms after 4 months.
Results demonstrate that the treatment with the L-arginine containing hair tonic led to an improvement in hair growth as well as the dermatological healthiness of the scalp already after a four months treatment period.

Discussion

Androgenetic alopecia (AGA) is a very common non-scarring disease that affects both, men and women [1]. As many pathogenic mechanisms considered to be involved in the onset of AGA, various treatment options are available. Topical and systemic acting drugs or ingredients, individually or combined, may be used and the choice depends on various factors including grading of AGA, efficacy, practicability, costs and risks [10].
In most cases on of the two currently approved drugs for treatment of AGA are used, topical minoxidil and oral finasteride (1 mg/day) ([21]). Due to the multifactorial etiology of AGA, other active principles are of interest for further investigation.

Mechanism of action

One approach is the topical use of L-arginine, which plays a role in many areas of human physiology, including the production of nitrogen monoxide (NO), a key molecule involved in vascular regulation, immune activity, and endocrine functions ([22]). L-Arginine is the substrate of the enzyme nitric oxide synthase (NOS), which is responsible for the production of nitrogen monoxide (NO) [11]. It acts as a signalling molecule in human dermal papilla cells and it is suggested that basal and androgen-mediated NO production is involved in the regulation of hair follicle activity [13].
Consequently, this leads to the hypothesis that L-arginine might be an interesting modulator of hair growth. To investigate how such a molecule may influence AGA and hair growth, a first step was to elucidate the basic amount of L-arginine in hair. Second step was to determine whether the L-arginine content in hair is affected by external application of an L-arginine containing hair tonic.

Furthermore, it was proposed that asymmetric dimethylarginine, (ADMA), an endogenous inhibitor of nitric oxide (NO) synthase, could serve as additional biomarker for inflammatory processes which may have an impact on skin function [23]. It is well known that oxidative stress, endothelial nitric oxide synthase (eNOS) inhibition, eNOS uncoupling, inflammation and shear stress play a pivotal role in ADMA pathophysiology [24]. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) synthesis, whereas L-arginine (Arg) and L-homoarginine (hArg) serve as substrates for NO synthesis. [25]

In the experiments described in this paper, we demonstrated that the application of an L-arginine containing hair tonic significantly increased the concentration of L-arginine in human subjects. Application of the hair tonic with the same chemical composition but without L-arginine did not change the L-arginine concentration significantly. Moreover, we were able to detect the competitive inhibitor of the NO synthase, asymmetrical dimethylarginine (ADMA) with analytical procedures in human hair.
Consequently, the data verified significant effect of the hair tonic on the content of L-arginine and ADMA in hair. The results suggest that the tonic acts as a hair-restorer influencing the local L-arginine and ADMA content.
The data support the hypothesis, that topically applied L-arginine has an impact on the activity of the NO synthase. An explanation could be that the blood supply of the hair papilla might be improved by an increased incorporation of L-arginine via a competitive displacement of ADMA with respect to the enzyme NO synthase. As a consequence, the level of local available nitric oxide increases and hair growth is stimulated. The L-arginine/ ADMA ratio may serve as a suitable parameter describing the situation of the NO synthase activity. Further in vitro studies are needed to elucidate the activity and expression of the NO synthase in the hair follicle and the impact of L-arginine on this process.

Observational studies

Three studies were performed to show the safety and efficacy of the L-arginine containing hair tonic. In all three studies with a treatment period of at least 4 months, a very good safety and tolerability was observed without any adverse effects on the skin.
Regarding efficacy, nearly 50% of the test subjects with ADA (study 2:9 from 19 test subjects = 47.3%; study 3: 40 of 82 = 48.7%) responded to the treatment with slight to significant increase in hair growth (density, quantity).

In one study, 40 test subjects reported that the hair growth was induced after several years of baldness. The analysis of the hair status revealed that in such cases hair growth was at

various stages and progressed after further treatment with the hair tonic. Hair growth at tonsures and receding hairlines suggest that a reactivation of hair roots had taken place.

In a study with female test persons a stop of hair loss was achieved in all persons analysed after 6 months (n=13). As the first goal of the hair tonic treatment is to reduce or stop the hair loss, the results could be judged as very successful.
In comparison to data available for drug treatments of androgenetic alopecia the presented approach has the great advantage of a very good local tolerance without any side effects as described for products containing finasteride or minoxidil, even after long term treatment.

A Cochrane analysis of oral finasteride revealed a moderate-quality evidence suggesting that daily use increases hair count but may generate side effects like the risk of sexual dysfunction [26]. Furthermore, oral finasteride has been shown to be effective in the treatment of hair loss in men at 1mg/day, while its efficacy in women has remained unclear [27]. Data suggest that a dosage of 2.5 mg/day or more may be effective in postmenopausal women in the absence of clinical or laboratory signs of hyperandrogenism. [28]

To generate a higher level of evidence regarding the efficacy of the product further controlled and randomized studies in a larger cohort are required. Nevertheless, the existing results presented indicate a valid and safe alternative to existing drug based topical and oral treatments of androgenetic alopecia.

Conclusion

L-arginine containing hair tonic solutions might be an easy to use and safe approach to treat test persons with androgenetic alopecia. Clinical studies with durations up to 18 months revealed that the continuous application of hair tonic

- increases the L-arginine concentration in hair and may have an impact on the NO-pathway
- results in a stop or delay of hair loss
- increases of hair density and volume
- and stimulates hair growth.

Evaluation of more than 140 probands demonstrated that the use of the tonic solution is safe without any side effects.

Thus, the results strongly suggest that the L-arginine hair tonic is capable to positively influence the attenuation of hair loss. Additional studies are needed to further elucidate the

clinical benefit of this treatment option. Complementary, synergistic interaction with other therapies might be evaluated further.

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