UniversitätsSpital Zürich
Dermatologische Klinik
Direktor: Prof. Dr. med. L. E. French

Arbeit unter Leitung von Prof. Dr. med. R.M. Trüeb

Randomisierte, doppelblind Placebo-kontrollierte Studie zur Wirksamkeit eines spezifischen, CYP (Cystin, Medizinalhefe, Pantothensäure)-Komplex-haltigen Arzneimittels zur Nahrungsergänzung (Pantogar) bei Frauen 60+ mit Haarausfall

INAUGURAL-DISSERTATION
zur Erlangung der Doktorwürde der Zahnmedizin
der Medizinischen Fakultät
der Universität Zürich

vorgelegt von
Barbara Hotzenköcherle Trüeb

Genehmigt auf Antrag von Prof. Dr. med. L. E. French
Zürich 2013

Contents

1. Abstract

Background: A former placebo-controlled trial demonstrated that administration of a L-cystine, medicinal yeast and pantothenic acid (CYP complex)-based dietary supplement improved anagen rates in women with telogen effluvium.

Objective: To verify efficacy of CYP complex in women aged 60 years or more.

Methods: A randomized, double-blind, placebo-controlled trial was conducted over 6 months in 36 healthy women suffering from telogen effluvium. Anagen rates were determined with digitalized epiluminiscence microscopy and digital imaging performed before, after 3 and 6 months of treatment.

Results: Both placebo and active compound showed significant improvement of mean anagen rates. Between the two groups these changes were statistically not significant. Inclusion of study patients in relation to seasonality of hair growth and shedding was inhomogeneous.

Conclusions: Despite prior evidence of efficacy of CYP-based nutritional supplement over placebo, superiority of the active compound over placebo was not demonstrated in this study. This observation was explained by inhomogeneous inclusion of study patients in relation to seasonality of hair growth and shedding. Depending on periodicity of hair growth and shedding, heterogeneity of patient inclusion may be enough to distort clinical efficacy results.

2. Introduction

Combining epiluminiscence microscopy with digital imaging has evolved as a simple to perform, noninvasive and reproducible method for the measurement of efficacy of hair growth promoting agents [1-4]. In a former double blind placebo-controlled study using the TrichoScan technique, a L-cystine, medicinal yeast and pantothenic acid (CYP complex)-based dietary supplement was demonstrated to positively influence hair growth in otherwise healthy women aged between 25 and 65 years complaining of hair loss, as opposed to placebo [5]. Since an open label pilot study with 5 women aged between 64 and 84 years again showed an increase of anagen rates within 3 months of treatment (unpublished data), we performed a double blind placebo controlled study with the aim to verify efficacy of CYP complex in women aged 60 years or more.

While both placebo and active compound showed significant improvement of mean anagen rates, between the two groups these changes were not significant. Herein we demonstrate that the impact of seasonality of hair growth and shedding and heterogeneity of patient inclusion in relation to the season were enough to distort clinical efficacy results.

3. Patients and Methods

Patients and Treatment

The trial was carried out as a single centre, randomized, double-blind, controlled, parallel group study to compare the efficacy of a CYP-complex based active compound with placebo in treating telogen effluvium in otherwise healthy women over a treatment duration of 6 months. Women aged 60 years or more were recruited through advertisement in the lay press.

Inclusion criteria were a history of increased hair loss, with or without clinical findings of female pattern hair loss (FPHL) Ludwig type I or II, and a centroparietal telogen hair rate > 20%, determined by the TrichoScan.

Exclusion criteria included: symptomatic diffuse alopecia (e.g. resulting from iron deficiency or thyroid gland disorder); FPHL Ludwig type III; androgenic alopecia with or without signs of virilization related to a history of polycystic ovaries, late onset adrenogenital syndrome, or tumours of the ovaries, adrenals or pituitary gland; systemic autoimmune diseases; wasting diseases (AIDS, malignant disease); alopecia areata; inflammatory scarring or other scarring alopecias; other inflammatory conditions affecting the scalp (seborrhoeic dermatitis, psoriasis, contact dermatitis); any treatment for hair loss or participation in another clinical trial within 3 months prior to entering the study; use of drugs that may cause hair loss (anticoagulants, lipid lowering drugs, retinoids, antiepileptics, beta-blocking agents, ACE inhibitors, antithyroid drugs, androgens, progestagens with partial androgenic effect, aromatase inhibitors, cytokines, cytotoxic drugs) within 3 months prior to entering the study; use of sulfonamide-containing drugs (interaction with p-

aminobenzoic acid or PABA); initiation or termination of hormone replacement therapy or hormonal contraception within 6 months prior to entering the study; any type of hormone replacement therapy or oral contraception containing a progestagen with androgenic effect (norethisterone, norgestrel, levonorgestrel, lynestrenol, tibolone); pregnancy or lactation; known hypersensitivity to lactose (placebo) or any component of the active compound.

The study was approved by the local Ethics Committee. If the patient was suitable for the study and had given her written informed consent, she was randomized into one of the two treatment arms and supplied with the active compound or placebo.

The composition of the active compound was as follows: 1 capsule active compound: L-cystine 20 mg, keratin 20 mg, medicinal yeast 100 mg, calcium pantothenate 60 mg, thiamine nitrate 60 mg, and PABA 20 mg. 1 capsule placebo: no active ingredient, lactose, microcrystalline cellulose, and magnesium stearate. The dosage was 1 capsule three times a day with meals, for the duration of the study (6 months).

Methods

The diagnosis of telogen effluvium was based on an increase of the telogen hair rate > 20% in the centroparietal scalp area determined by the TrichoScan, and careful exclusion of other causes of hair loss. This included an in-depth history and clinical examination related to the begin and duration of hair loss, as well as its pattern. A careful personal history of diet, illness, operations, medications, including hormones. The following laboratory screening tests were performed (normal ranges): CRP (< 5 mg/l), ferritin (> 10 μg/l), basal TSH (0.27-4.20 mU/l).

To determine the telogen hair rate for inclusion, and the anagen hair rate throughout the study, an area of 1.8 cm^2 was defined in the centroparietal scalp using a stencil template (diameter 16 mm). In that test zone the hair was clipped (Hairliner, Wella Germany). All clipped areas were marked with a central, single red tattoo. The tattoo was visible throughout the study. Gray or fair hairs have only limited contrast in comparison to the scalp. Therefore, the clipped hairs within the target area were dyed with a commercially available solution (RefectoCil®, Gschwentner, Vienna, Austria). Thereafter, the colored area was cleansed with an alcoholic solution (Kodan® Spray, Schülke & Mayr, Vienna, Austria) and digital images were obtained at 20-fold (analyzed area: 0.62 cm^2) magnification by means of a digital ELM system (Fotofinder DERMA, Teachscreen Software, Bad Birnbach, Germany) while the area was still wet. This digital camera is equipped with a rigid contact lens which ensures that the images are always taken at the same distance from the scalp. Images were taken at day zero immediately after clipping, and three days after clipping, and three and six months after the initial visit, respectively. For measurement of anagen hair rated a commercially available software (TrichoScan) developed specially for this purpose was used (DatInf, Tübingen) [1].

The measures of outcome were the anagen hair rate at baseline, after 3 and 6 months of treatment.

Randomization of patients was done with RANCODE, version 3.6, including 45 patients. Treatment 1 was verum, treatment 2 placebo at a ratio of 1:1, no stratification.

Statistics

Patients receiving active compound were compared with placebo at baseline (T = 0), 3 months (T = 3) and 6 months (T = 6). Repeated measures ANOVA with Greenhouse – Geisser correction was performed to analyze the anagen rates (AR) in % over time. Changes of AR over 6 months were compared between the two groups using the unpaired t-test.

4. Results

A total of 45 patients were enrolled. 36 completed the study: 15 in the active compound group, 21 in the placebo group. Nine patients withdrew from the trial due to: intercurrent unrelated illness (3, 2 on active compound and 1 on placebo), use of drugs that may cause hair loss (3, all on active compound), gastrointestinal upset (2, 1 on active compound, 1 on placebo), and initiation of other treatment for hair loss (other dietary supplement, 1 on placebo).

The age range of the women who completed the study was 60 - 82 years (mean: 68 years), in the active compound group 60 – 78 (mean: 68 years), in the placebo group 60 – 82 years (mean: 68 years).

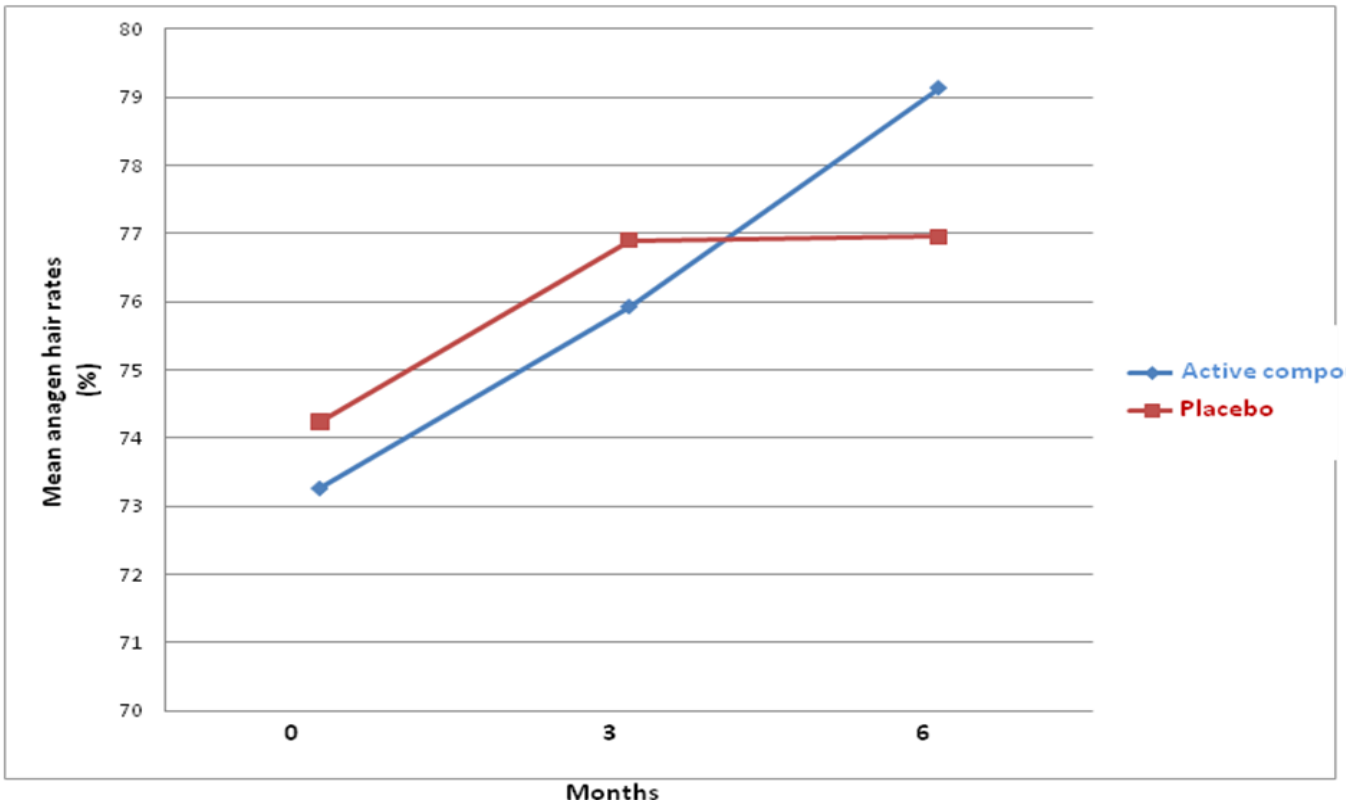
The results of the anagen hair rates (in %) at baseline, 3, and 6 months are presented in terms of descriptive statistics in **Table 1** and showed graphically in **Figure 1**. There was an overall significant increase AR % over time ($p < 0.001$), with no significant interaction between time and treatment ($p = 0.19$). The overall difference between the two groups was not significant ($p = 0.97$). The changes of AR in % between the two groups over 6 months were not significant ($p = 0.12$).

The active compound was generally well tolerated: 1 patient reported gastrointestinal symptoms.

Table 1. Results of anagen hair rates (%)

	Active compound					Placebo				
	N	AR % Min.	AR % Max.	AR % Mean	Std.D.	N	AR % Min.	AR % Max.	AR % Mean	Std. D.
T= 0 months	15	62	79	73.3	6.1	21	58	79	74.2	6.5
T= 3 months	15	62	86	75.9	6.6	21	52	93	76.9	9.0
T= 6 months	15	63	88	79.1	6.6	21	44	90	77.0	9.9

Figure 1. Improvement of anagen rates after treatment



5. Discussion

The hair follicle is subject to constant turnover in the course of perpetual cycles through phases of proliferation during anagen, involution during catagen and resting during telogen, with regeneration in the successive hair cycle. In telogen, the hair shaft matures into a club hair, which is held tightly in the bulbous base of the follicular epithelium, before it is eventually shed during teloptosis [6,7]. Cyclic hair growth activity occurs in a random mosaic pattern with each follicle possessing its own individual control mechanism over the evolution and triggering of the successive phases. Systemic factors, such as the hormonal system, cytokines and growth factors, as well as external factors linked to the environment, toxins, deficiencies of nutrients, vitamins and energy, have influence.

In general, the pathological dynamics of hair loss can be related to disorders of hair cycling [8]. Whatever the cause, the follicle tends to behave in a similar way, with telogen effluvium representing the most frequent cause of hair loss [9].

Telogen effluvium results from a disruption of the hair cycle resulting in increased proportion and shedding of telogen hair (> 15%). Hair loss usually affects less than 50% of scalp hair and presents with diffuse thinning of the hair, that is most conspicuous at the temples, and a positive hair pull test of telogen club hairs.

Headington [10] proposed a classification of telogen effluvium into different functional types based on changes in the different phases of the hair cycle. Telogen effluvium may either result from synchronization phenomena of the hair cycle with an increase in shedding of hairs from the telogen phase of the cycle, or from shortening of the

duration of the anagen phase. While synchronization phenomena underlie diffuse telogen effluvium, shortening of the duration of anagen underlies androgenetic alopecia. Finally, Headington also suggested that a delayed telogen release underlies moulting in mammals, and possibly mild telogen effluvia in humans following travel from low-daylight to high-daylight conditions. In this case, hair follicles remain in a prolonged telogen phase rather than being shed and recycling into anagen. When finally teloptosis sets in, again the clinical sign of increased shedding of club hairs is observed.

In a former study of 823 otherwise healthy women with telogen effluvium during an observational period of 6 years, the existence of an overall annual periodicity in the growth and shedding of hair was demonstrated, manifested by a maximal proportion of telogen hairs in July [11]. Taking a scalp hair telogen phase duration of approximately 100 days into account, one would expect shedding of these hairs by autumn. A second peak seems to exist, although less pronounced, in April. These findings confirmed the results of prior studies performed in smaller populations and male subjects [12,13]. It was pointed out that the existence of seasonal fluctuations in hair growth and shedding would potentially complicate the assessment of pharmacological effects, and therefore would have serious implications for investigations with hair-growth-promoting agents: depending on the stage of periodicity in growth and shedding of hair for a particular subject, the heterogeneity of included subjects may be enough to distort the clinical efficacy results and the perceived benefit of an investigational agent. In the active stage of seasonal telogen effluvium, the involved hair follicles would probably fail to respond to the therapeutic agent, which may cause a false-negative result. In the recovery stage, the increased

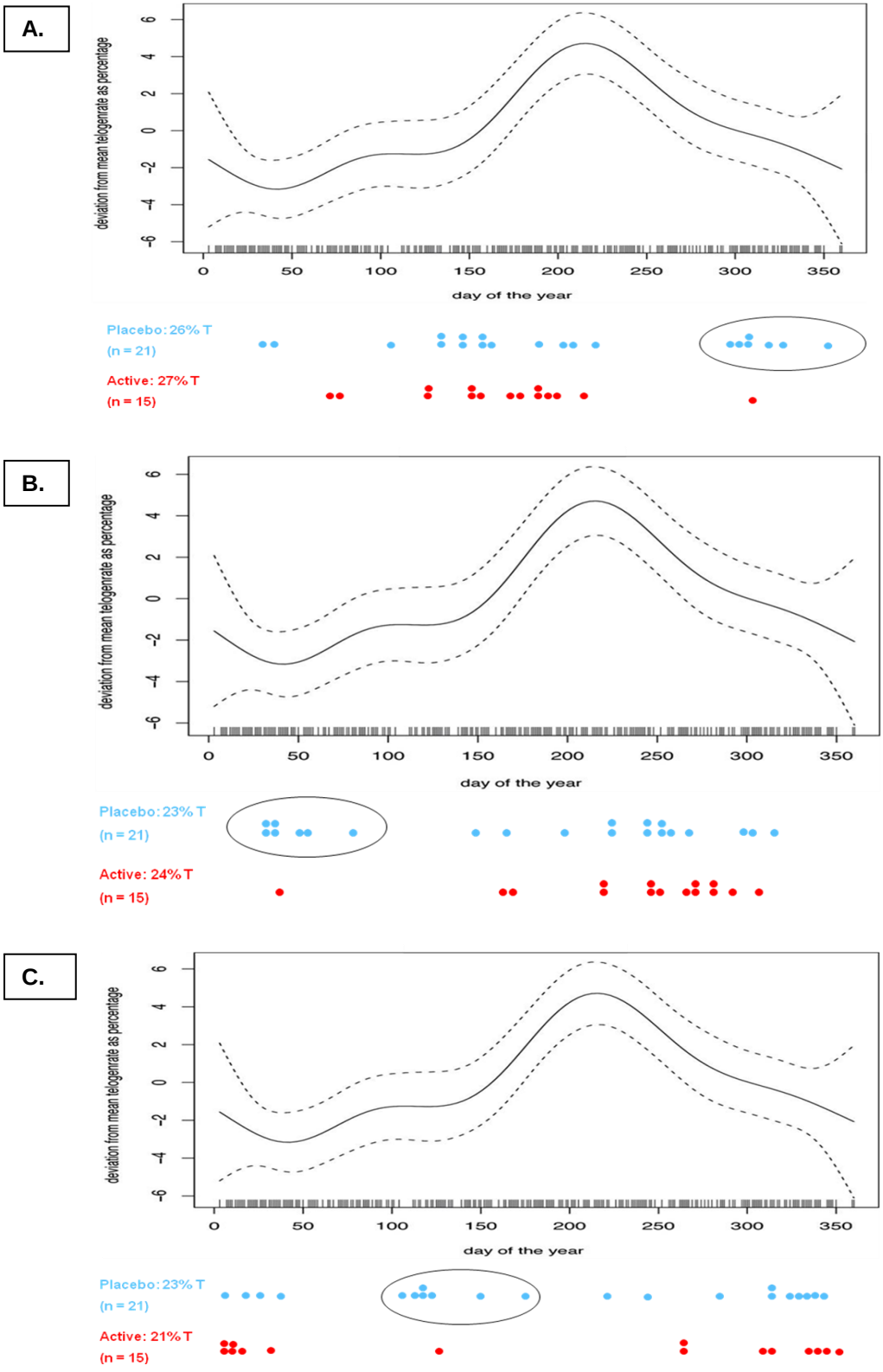
amounts of spontaneous hair regrowth might be interpreted falsely as a positive result.

In the present study, both placebo and active compound showed significant improvement of mean anagen rates within the 6 months of treatment. Despite prior evidence of efficacy of CYP-based nutritional supplement over placebo [5], the changes of anagen rates between the two groups were not significant.

This observation was explained by inhomogeneous inclusion of study patients in relation to seasonality of hair growth and shedding (**Fig. 2 A-C**): In the placebo group a cluster of 7 patients (versus 1 patient in the active compound group) were included, that profited from seasonal hair growth within the first 3 months of the study. From 3 to 6 months the AR in % in the placebo group flattened, while in the active compound group the AR in % further increased and exceeded the placebo group (**Fig. 1**).

We conclude that the impact of seasonality of hair growth and shedding must be taken into consideration in clinical trials with hair growth promoting agents, especially in studies with small numbers of patients and short study durations. Care should be taken to maximize homogeneous patient inclusion in relation to the season.

Figure 2. Inhomogeneous inclusion of study patients in relation to periodicity of hair growth and shedding: A. at inclusion, B. at 3 months, C. at 6 months. Ellipse highlights cluster of patients in the placebo group that profited from seasonal hair growth within first 3 months.



6. References

1. Hoffmann R. TrichoScan: a novel tool for the analysis of hair growth in vivo. J Investig Dermatol Symp Proc. 2003;8:109-15.

2. Hoffmann R. Trichoscan: what is new? Dermatology. 2005;211:54-62.

3. Hoffmann R. TrichoScan: combining epiluminescence microscopy with digital image analysis for the measurement of hair growth in vivo. Eur J Dermatol. 2001;11:362-8.

4. Hoffmann R. TrichoScan, a GCP-validated tool to measure hair growth. J Eur Acad Dermatol Venereol. 2008;22:132-4

5. Lengg N, Heidecker B, Seifert B, Trüeb RM. Dietary supplement increases anagen hair rate in women with telogen effluvium: results of a double-blind, placebo-controlled trial. Therapy 2007;4:59-65

6. Paus R, Cotsarelis G. The biology of hair follicles. N Engl J Med 1999; 341: 491–497.

7. Piérard-Franchimont C, Piérard GE. Teloptosis, a turning point in hair shedding biorhythms. Dermatology 2001; 203: 115–117.

8. Paus R. Control of the hair cycle and hair diseases as cycling disorders. Curr Opin Dermatol 1996; 3: 248–258.

9. Kligman AM. Pathologic dynamics of human hair loss. I. Telogen effluvium. Arch Dermatol 1961; 83: 175–198.

10. Headington JT. Telogen effluvium: new concepts and review. Arch Dermatol 1993; 129: 356–363.

11. Kunz M, Seifert B, Trüeb RM. Seasonality of hair shedding in healthy women complaining of hair loss. Dermatology. 2009;219:105-10.

12. Randall VA, Ebling FJG. Seasonal changes in human hair growth. Br J Dermatol 1991;124:146-51

13. Courtois M, Loussouarn G, Hourseau S, Grollier JF. Periodicity in the growth and shedding of hair. Br J Dermatol 1996;134:47-5

7. Acknowledgments

I am grateful to the following people who have been part of writing this dissertation and supporting my work. First of all to Professor Ralph M. Trüeb, I extend my deepest gratitude for leading the way to this project and for being very supporting, helping and patient. My thanks to Doctor Barbara Heidecker , for her efficiency in the work with the patients. Professor Burkhardt Seifert did all the statistics, I am very grateful for his interpretations and the patience with which he answered all my questions.

The study was possible with support from Merz Pharmaceuticals GmbH, Germany.