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A spermidine-based nutritional supplement prolongs the anagen phase of hair follicles in humans: a randomized, placebo-controlled, double-blind study

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ABSTRACT

Background: Spermidine has been shown both in vitro and in mice models to have an anagen-prolonging effect on hair follicles (HFs).

Objectives: To evaluate the effects of a spermidine-based nutritional supplement on the anagen phase of HFs in healthy human subjects in a randomized, double-blind, placebo-controlled trial.

Methods: One hundred healthy males and females were randomized to receive a tablet containing a spermidine-based nutritional supplement or a placebo once daily for 90 days. At the beginning and the end of the treatment period, 100 HFs were plucked and subjected to microscopic evaluation to determine the number of anagen V-VI HFs, and immunohistochemical examination was performed to quantify the Ki-67 and c-Kit levels in the hair bulbs. Pull test was performed after three and six months.

Results: The spermidine-based nutritional supplement increased the number of anagen V-VI HFs after three months of treatment, accompanied by increased Ki-67, a marker for cellular proliferation, and decreased c-Kit, a marker for apoptosis, levels. All results were also significantly better when compared to the placebo group. The pull test remained negative after six months in all patients receiving the spermidine supplement, while 68% of the subjects in the placebo group had a positive pull test.

Conclusions: This preliminary study shows that a spermidine-based nutritional supplement can prolong the anagen phase in humans, and therefore might be beneficial for hair loss conditions. Further studies are needed to evaluate its effects in specific different clinical settings.

Introduction

The polyamines, consisting of putrescine, spermidine and spermine, are straight chain aliphatic compounds that are ubiquitously found in living organisms [1,2]. Their levels are strictly controlled by complex metabolic pathways incorporating polyamine biosynthesis, catabolism, and transport [3,4]. They are essential for the survival and growth of eukaryotic cells by regulating gene expression and protein synthesis, affecting a large variety of cellular processes, including cell growth, differentiation, and regulation [5-7].

The hair follicle (HF), one of the most highly proliferative organs in mammals, has also been shown to be dependent on polyamines for its normal growth, function and cycling. This has been demonstrated in several mouse models, where changes in polyamine metabolism led to hair loss due to alteration in the proliferation of the HF keratinocytes [8-18]. Spermidine, the prototypic polyamine in humans, is especially important for normal hair growth. Indeed, topical administration of eflornithine, which inhibits ornithine decarboxylase, the rate-limiting enzyme in the biosynthesis pathway of polyamines, is used to treat excessive hair growth in females [17,18]. It has also been shown to decrease the anagen phase and induce apoptosis in human HFs in vitro [19]. Spermidine and its metabolically stable analog, N^1-methylspermidine, were demonstrated to prolong anagen and affect epithelial stem cell functions in human HFs in vitro [5,20,21]. In vivo, topical application of α-methylspermidine, a stable analogue of spermidine, enhanced hair growth in telogen phase mice [22]. Previous preliminary studies have shown the effectiveness of spermidine-containing nutritional supplements for the treatment of telogen effluvium [23,24]. However, the effect of spermidine on the anagen phase in normal subjects has never been studied in humans. Therefore, we conducted a randomized, double-blind, placebo-controlled trial on 100 patients, to evaluate the effects of a spermidine-based nutritional supplement on the anagen phase of HFs in healthy human subjects.

Patients and Methods

Subjects

A total of 100 healthy men and women were recruited into the study after giving written consent. The participants were randomized into the treatment group (31 men and 19 women, 36.08 years of age on average, age range 23-50) or placebo group (36 men and 14 women, 35.6 years of age on average, age range 22-48). All participants in the study had unremarkable dietary and lifestyle habits. Exclusion criteria included initial signs of androgenetic alopecia demonstrated by miniaturization of the hair shaft as observed in the occipital region; a family history of androgenetic alopecia; congenital or acquired diseases affecting the hair shaft; the use of any topical and/or systemic therapy for hair loss in the previous three months; regular treatment with corticosteroids, hormone therapies, anti-androgenic acting products (e.g., spironolactone, cimetidine, ketoconazole) or anticoagulants; infections or other active disease up to three months prior to beginning the study; organic diseases affecting the kidneys, liver, cardiovascular system, lungs or the central nervous system; diabetes mellitus; alcohol or recreational drug abuse in the year preceding the start of the study; and clinical history of sensitivity or allergic reaction. All patients were evaluated and enrolled to the study in the Rinaldi Dermatologic Clinic, Milan, Italy.

Study design

This study was a single center, parallel group, double-blinded, randomized, placebo-controlled trial with 1:1 allocation to treatment groups. All subjects signed an informed consent form in accordance with the ICH and Good Clinical Practice (GCP) Guidelines, prior to undergoing any study related procedures. Each patient was randomly allocated to either of two groups (n = 50 in each group): a treatment group receiving a tablet containing a spermidine-based nutritional supplement, taken once daily after the main meal, and a placebo group. The treatment was given for 90 days.

Assessment criteria

All patients were evaluated at three time points: T0 = beginning of the study, T1 = 3 months after the beginning of the study, and T2 = 6 months after the beginning of the study. Identification of HF lifecycle phase by means of epiluminescence imaging (trichogram to verify normal cycling status, based on the presence of anagen for at least 80% of hair follicles) was performed at T0 and T1. At both visits, 100 hair bulbs were plucked from the occipital area of all subjects. The occipital area was chosen because hair bulbs in this area are not affected by androgen receptor changes typical of androgenetic alopecia, thus avoiding enrollment into the study of subjects suffering from as yet clinically undetectable androgenetic alopecia. The plucked hair bulbs were immersed in saline solution and evaluated microscopically to select anagen phase V–VI HFs (differentiating from previous phases and above all from initial catagen phase hair bulbs). Standardized parameters reported and proposed by Kloepper et al. were used [19]. The levels of Ki-67, a marker of cellular proliferation, and of c-Kit, a marker of apoptosis, were determined immunohistochemically on the plucked HFs as described previously [25]. The extent of hair loss was also assessed by the hair pull test on T0, T1 and again on T2 to check for possible onset of physiological telogen effluvium, typical of the autumn season [26].

TABLE 1. Number of anagen phase V-VI hair bulbs

	Placebo Mean (s.d.) N=50	Spermidine Mean (s.d.) N=50	P value (between treatment groups, Student's t-test)
T0	25.54 (4.05)	24.64 (4.45)	0.29 (n.s.)
T1	20.24 (3.14)	37.44 (3.84)	
Absolute change between T1 and T0	-5.3 (2.3)*	12.8 (6.87)*	<0.0001

*p<0.0001 within treatment group, change from T0, paired t-test.
n.s. non significant; s.d. standard deviation.

TABLE 2. Expression of Ki-67 and c-Kit

	Placebo Mean (s.d.) N=50	Spermidine Mean (s.d.) N=50	P value (between treatment groups, Student's t-test)
Ki-67			
T0	91.58 (8.83)	90.08 (12.12)	0.48 (n.s.)
T1	86.63 (7.66)	102.77 (10.75)	
Absolute change between T1 and T0	-4.96 (6.76)*	12.69 (8.1)*	<0.0001
c-Kit			
T0	9.19 (1.08)	9.67 (1.12)	0.03
T1	10.99 (1.14)	7.52 (1.22)	
Absolute change between T1 and T0	1.8 (1.07)*	-2.16 (1.24)*	<0.0001

*p<0.0001 within treatment group, change from T0, paired t-test.
n.s. non significant; s.d. standard deviation.

Student's *t* test was used for comparing the number of anagen hair bulbs and Ki-67 and c-Kit levels at baseline and at T1, and the change from baseline was compared between groups using the paired t-test. Comparison of the pull test results between T1 and T2 was performed using the Chi-square test or Fisher's exact test. The statistical analyses were performed on all subjects enrolled (n=100) by means of a two-tailed test and on a significance level of 0.05 (p-value).

Results

Number of anagen V-VI hair bulbs

The number of anagen hair bulbs increased in the spermidine treatment group between T0 and T1 (more than 50% increase), while in the placebo group there was a significant decrease in the number of anagen hair bulbs of approximately 20% (Table 1). There was a highly statistically significant difference in the change in anagen hair bulb number between the spermidine-treated group and the placebo group (p<0.0001).

Ki-67 and c-Kit assessments

Treatment with spermidine increased the levels of the proliferation marker Ki-67 after 3 months of treatment, accompanied by decreased levels of the apoptosis marker, c-Kit (Table 2). At the same time, in the placebo group, Ki-67 levels were decreased and c-Kit levels increased. There was a statistically significant difference between the spermidine-treated group and the placebo group.

Pull test

At baseline, all subjects had a negative pull test (Table 3). There was a gradual increase in the number of subjects that had a positive pull test in the placebo group, with 14 subjects at T1 (28%), and 34 subjects at T2 (68%) having a positive test (Table 3). This was in contrast to the subjects in the spermidine-treated group, where only one subject was found to have a positive test at T1 (Table 3), and none at T2. The difference between the groups was statistically significant at both time points.

Discussion

Our results provide preliminary evidence that a spermidine-based nutritional supplement, when given orally once daily for 90 days, can promote anagen prolongation, and reverse the transition between anagen to catagen and to telogen. Part

TABLE 3. Pull test results

	T0		T1		T2	
	Placebo N (%)	Spermidine N (%)	Placebo N (%)	Spermidine N (%)	Placebo N (%)	Spermidine N (%)
-	50	50	36 (72)	49 (98)*	16 (32)	50 (100)#
+	-	-	14 (28)	1 (2)*	19 (38)	-
++	-	-	-	-	12 (24)	-
+++	-	-	-	-	3 (6)	-

*p<0.0001 by Fisher's exact test; #p<0.0001 by Chi-Square test

of these effects could probably be attributed to an increased proliferation and decreased apoptosis in the hair bulb cells, as assessed by determining Ki-67 and c-Kit levels.

The potential beneficial effects of spermidine on human HFs have been suggested previously, based on several mice model studies [27]. This assumption has received additional support from two recent in vitro studies, using human HF organ cultures and cell cultures [20,21]. In the first study, spermidine was found to enhance hair shaft elongation and prolong anagen, accompanied by increased human hair matrix and epidermal keratinocyte proliferation [21]. The anti-apoptotic and anagen-promoting effects of spermidine were recapitulated when N^1-methylspermidine, a metabolically stable spermidine, was used [20]. The relevance of the human HF culture model for polyamines research has been demonstrated previously, when eflornithine, which is being used in clinical practice for the treatment of excess facial hair growth [17,18], also induced catagen in vitro [19].

The exact mechanism by which spermidine exerts its beneficial effects on the human HF is still not entirely clear. It has been shown previously that spermidine can differentially modulate the gene expression profile of HFs, which can have functional relevance to human hair growth and cycle [21]. Furthermore, N^1-methylspermidine can exert anti-oxidative and anti-inflammatory effects on human cells in vitro [20], which are both relevant to the human HF growth and function. Polyamines, and among them spermidine, might also be linked to the hairless protein [28,29], which has an important role in controlling normal hair function and cycle [20,31]. The fact that spermidine was found to enhance longevity supports the anagen-promoting effects observed in this study, as the duration of anagen is a good indicator for the HF vitality [32-34].

This study shows that oral spermidine can enhance anagen prolongation in humans. Anagen prolongation has significant clinical implications for different hair disorders, as it directly affects the amount of hair that is shed and therefore the number of HFs located on the scalp. Furthermore, although spermidine-containing tablets were given for only 90 days, the effect was still evident at least three months after the last administration of the pill, as demonstrated by the negative pull test in the treatment group. These preliminary results can serve as a proof of principle to the fact that oral spermidine can exert functional effects on human HFs and further strengthen previous results that showed its effectiveness for the treatment of telogen effluvium [23,24]. The possible beneficial effects of this compound for telogen effluvium and other hair disorders, such as pattern hair loss, need to be further confirmed in larger controlled studies.

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