The value of Triphasic®, a combination of ATP and Biotrinine for the treatment of progressive hair loss

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Introduction

Progressive hair loss is more common in men (30% at 30 years old and 50% from 50 years old) than in women, but women are nevertheless affected, particularly after the menopause (25% between 35 and 45 years old and 40% in women over 50) [1, 2]. Hair loss is associated with heredity, hormonal factors, and age and is more diffuse in women, and may ultimately lead to complete baldness in men if left untreated. This may have a major psychological impact in terms of self-image due to the changes it causes.

Recent advances in understanding the detailed mechanisms of progressive hair loss now enable us to offer effective treatments. The lifespan of hair is categorised into three phases: anagen (growth phase), catagen (resting phase), and telegon (hair loss phase). The aim of any treatment is to prevent this hair growth cycle from being shortened. However, we now have a better understanding of the highly regulated process of the hair growth cycle as well as the way in which the complex molecular mechanisms involved are implemented, particularly different growth factors and Wnt/ß-catenin signalling, which are thus the therapeutic targets of choice [3-5].

Molecular effects of Biotrinine

Biotrinine is a new active agent extracted from cress (*Nasturtium officinale*) and nasturtium (*Tropaeolum majus*). Its pharmacological effect, in synergy with ATP, has been demonstrated *in vitro*.

Activation of the Wnt/ß-catenin signalling pathway

Activation of the Wnt/ß-catenin signalling pathway in the dermal papilla and epithelial stem cells in the bulge is a key element in supporting proliferation and differentiation of follicular keratinocytes (the start and maintenance of the anagen phase) [6, 7]. It should be stressed that androgens suppress the differentiation of stem cells in the hair follicle by inhibiting Wnt signalling in androgenic alopecia [5].

The Dickkopf-related protein 1 (DKK1) hair growth inhibitor blocks activation of the Wnt/ß-catenin signalling pathway and promotes progression of the catagen phase [8]. Androgens stimulate the expression of DKK1 [9].

In vitro, hair dermal papilla cells (HDPC) incubated with Biotrinine demonstrated a significant increase in activation of the Wnt/ß-catenin pathway and a decrease in the production of DKK1 ($p<0.05$ and $p<0.01$, respectively), in comparison with controls *(figures 1A and B)* [10, 11].

Activation of growth factor synthesis

Keratinocyte Growth Factor (KGF) and Hepatocyte Growth Factor (HGF) are produced by the dermal papilla. KGF stimulates hair stem growth and, with HGF, maintains the anagen phase [12, 13].

In vitro, HDPC incubated with Biotrinine demonstrated a significant increase ($p<0.01$) in the production of HGF in comparison with controls, but KGF was unchanged. ATP alone did not lead to a significant increase in HGF, while KGF was significantly increased ($p<0.01$).

The combination of ATP and Biotrinine led to a significant increase in the production of HGF and KGF ($p<0.01$). The effect of the two combined was significantly higher than that of each of the components separately, thus demonstrating a synergetic effect *(figures 2A and B)* [14].

Activation of aromatase expression

Androgenic alopecia is dependent upon androgens [15, 16]. The aromatase enzyme, encoded by the *CYP19A1* gene, counters the effects of testosterone by catalysing its conversion into oestrogen, 17-ß-estradiol.

An *in vitro* study was launched to evaluate the effect of Biotrinine on *CYP19A1* gene expression (mRNA) and aromatase protein expression. HDPC incubated with Biotrinine demonstrated activation of expression of the *CYP19A1* gene by a factor of two, and a

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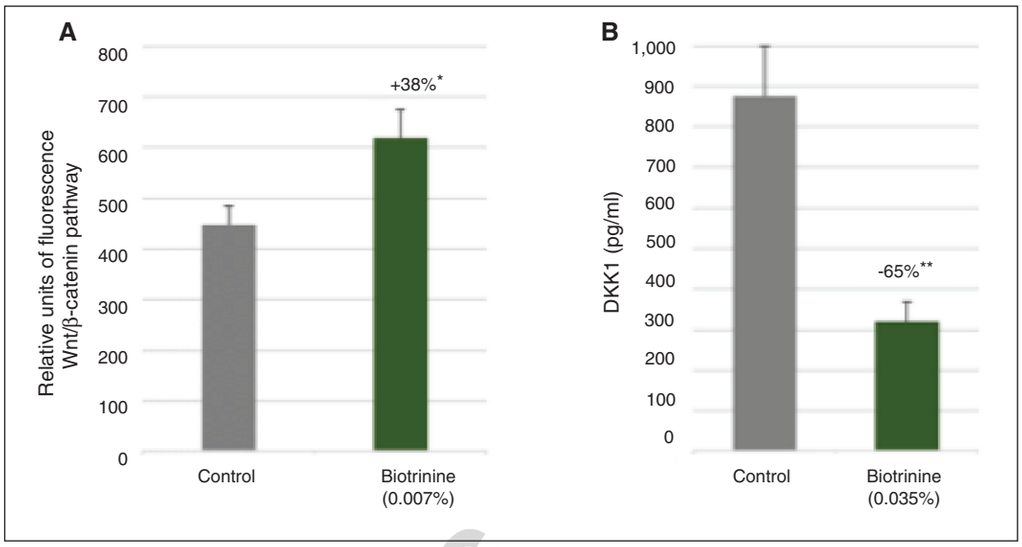

Figure 1. A) Activation of Wnt/ß-catenin in HDPC (for further details, refer to [10]). The HDPC were transfected by a lentivirus expressing the luciferase gene under the control of the TCF/LEF promoter. The transduced cells were incubated for 24 hours with Biotrinine dry extract before expression of luciferase was measured (n=3 donors). $^{*}p<0.05$ compared to control (Dunnett test). B) Production and release of DKK1 by HDPC (for further details, refer to [11]). Expression of the DKK1 protein was measured in supernatants from HDPC culture (ELISA) after 24 hours of incubation with Biotrinine dry extract (n=4 donors). $^{**}p<0.01$ compared to control (Dunnett test).

significant increase in the level of expression of aromatase protein ($p<0.05$) (*figure 3*). [17].

The various preclinical studies presented here have thus made it possible to specify the mode of action of Biotrinine, which acts on each of the key stages of the growth cycle of hair by:

- stimulating initiation of the anagen phase;
- supporting maintenance of the anagen phase;
- delaying progression of the catagen phase.

Biotrinine is thus a good candidate for treating chronic hair loss and may be combined with ATP with a view to potentiating their respective effects.

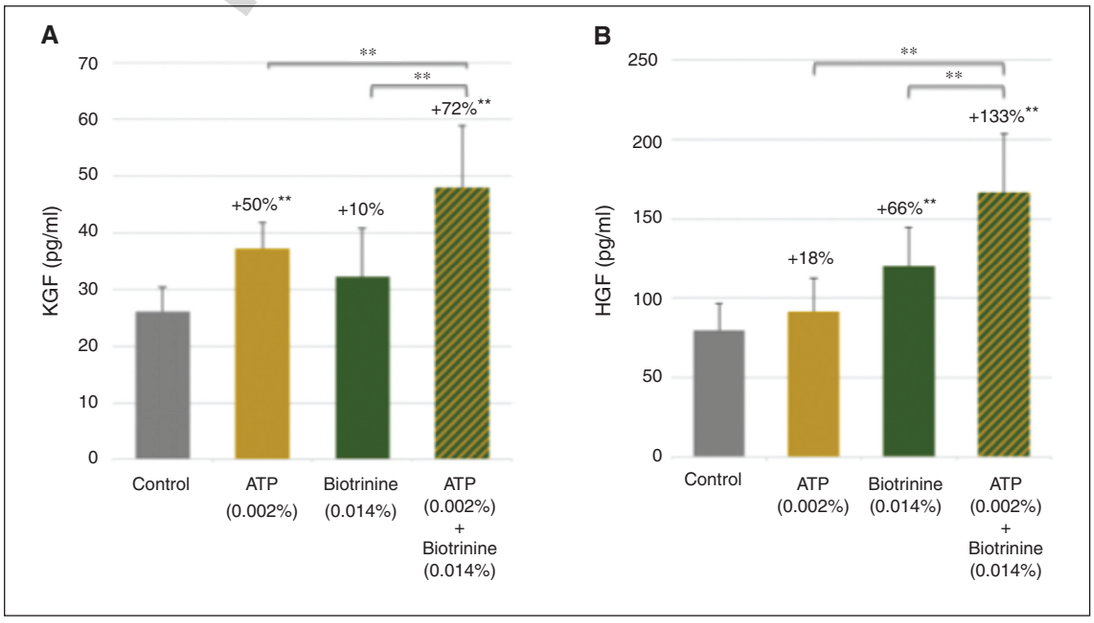

Figure 2. A) Production and release of KGF by HDPC. B) Production and release of HGF by HDPC. The expression rate(s) of KGF and HGF growth factors was measured in supernatants from HDPC culture (A: ELISA; B: Luminex) after 24 hours of incubation with Biotrinine, ATP, and a combination of the two (n=3 donors). ** $p<0.01$ compared to control (Dunnett test) (for further details, refer to [14]).

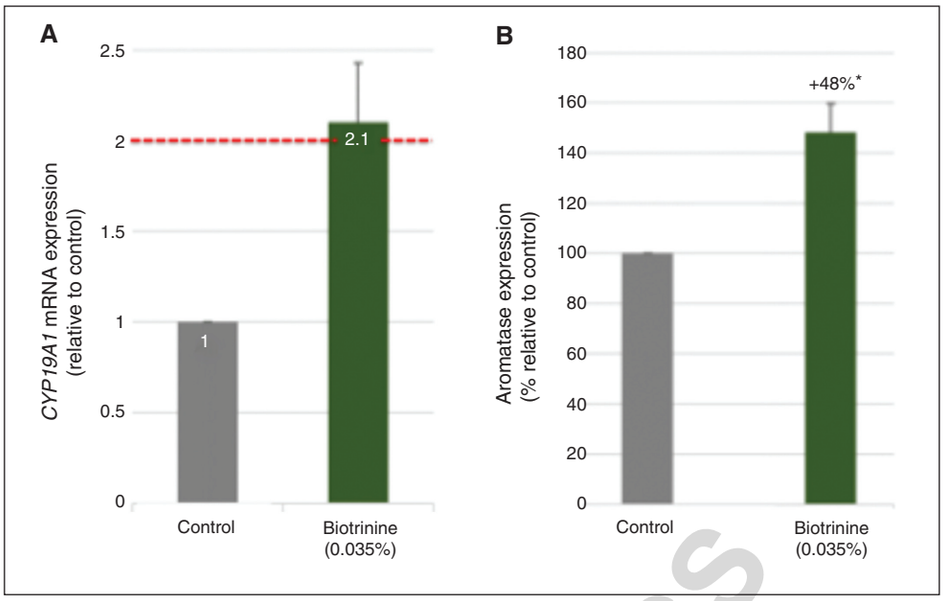

Figure 3. Expression of the *CYP19A1* gene in HDPC. A) The rate of expression of the aromatase gene was measured in HDPC after 24 hours of incubation with Biotrinine (n=4 donors). B) Expression of aromatase in HDPC. The rate of expression of aromatase was measured in HDPC (by western blot) after 24 hours of incubation with Biotrinine (n=3 donors). $^{*}p<0.05$ compared to control (Tukey test) (for further details, refer to [17]).

Triphasic® serum: demonstration of clinical effectiveness

Triphasic® serum is a combination of different active ingredients, more specifically, naturally occurring Biotrinine and ATP. It is produced in three forms (aqueous, oil and powder), mixed extemporaneously prior to application.

Methods and subjects

A clinical study of tolerance and effectiveness was carried out on 63 subjects affected by androgenic alopecia (30 women and 33 men) with an average age of 38 years [18]. Alopecia was quantified according to the Ludwig scale for women (four stages from onset to advanced) and the modified Norwood-Hamilton scale for men (seven stages from onset to complete baldness of the head and frontal area). The population included 16 women in Stage I, 13 women in Stage II, and one woman in Stage III, and nine men in Stage II, 14 men in Stage III, and ten men in Stage IV. All proceeded with two applications per week for one month followed by one application per week for two months.

Tolerance and effectiveness were assessed on Day (D) 1, Month (M)1, M2 and M3 by:

– Phototrichogram: the reference method which consists of a measure of surface units of total density, the percentage of anagen hairs, and the speed of growth based on a comparison of photographs taken at time intervals of a specific area of the scalp.

– Clinical notation of the overall effect: the investigator used macrophotographs of the frontal area and the vertex to obtain a score based on three parameters (hair density and volume, and width of the centre parting) on a scale of 0 to 10.

– Self-assessment of hair quality according to measurement of three parameters (hair density, volume, and thickness) on a scale of 0 to 10.

Results

Skin tolerance was good.

After three months of treatment, results of the phototrichogram showed a significant increase of 7,490 hairs across the entire scalp, including 2,996 in the alopecia area in men (*figure 4*).

Clinical measurement of hair density significantly improved with a 19% increase ($p<0.0005$) in the entire study population (n=60), 14% ($p<0.0005$) in men (n=31), and 24% ($p<0.0005$) in women (n=29).

Self-assessed measurement of hair density significantly improved with a 34% increase ($p<0.0005$) in the overall study population (n=60), 23% ($p<0.0005$) in men (n=31), and 46% ($p<0.0005$) in women (n=29).

The clinical score of overall effectiveness significantly improved with a 13% increase ($p<0.0005$) in the overall study population (n=60), 12% ($p<0.0005$) in men (n=31), and 14% ($p<0.0005$) in women (n=29).

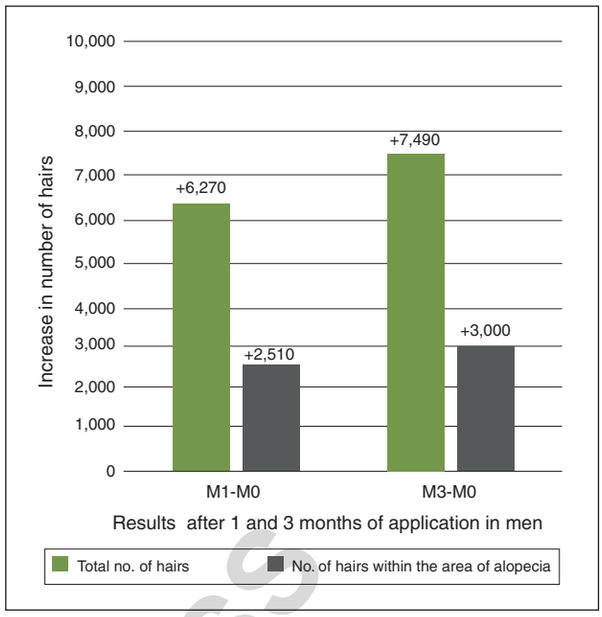

Figure 4. Phototrichogram showing an increase in the total number of hairs and hairs in the alopecia area at three months in male subjects (for further details, refer to [18]).

Stimulation of skin microcirculation using the new applicator nozzle

A study was carried out to compare the effect of the new Triphasic® applicator nozzle compared to the classic applicator nozzle. In this intra-individual open study, cutaneous microcirculation was measured using TiVi600® (a tissue viability imager) before and after a single application [19]. The results obtained showed that the use of the new applicator nozzle significantly stimulated cutaneous microcirculation with an average increase of 12% (p=0.001) (*figure 5*). The classic applicator nozzle had no statistical effect on stimulation of cutaneous microcirculation.

Conclusion

Triphasic®, a combination of active agents, notably, naturally occurring Biotrinine and ATP (patent pending[1]), acts at each decisive stage of the hair life cycle during the anagen phase, involving:

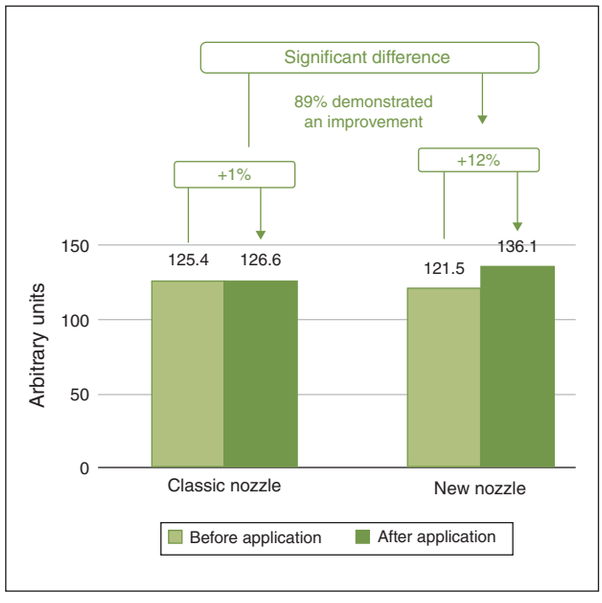

Figure 5. Stimulation of skin microcirculation using the new applicator. $^{*}p<0.05$ *vs T0* (for further details, refer to [19]).

– Activation of Wnt/ß-catenin signalling required for hair growth at birth.
– Stimulation and extension of growth reflected in clinical settings by re-densification of the hair.
– Prolongation of the maturity stage, delaying hair loss and preserving hair thickness.

The clinical effectiveness of the original Triphasic® serum formula was demonstrated in a reference study on progressive hair loss using a rigorous protocol.

Three months of treatment, consisting of two applications per week in the first month and one application per week in the next two months, led to a significant improvement in hair thickness, objectively assessed by phototrichogram in men and self-assessed in subjects of both sexes. The treatment led to an overall improvement in subjects of both sexes, as assessed by the investigator.

Key points

- Biotrinine acts on the key stages of hair growth by stimulating initiation of the anagen phase (growth phase), supporting the maintenance of this phase, and delaying progression of the catagen phase (resting phase).
- The combination of Biotrinine and ATP leads to a potentiation of their respective effects.
- Triphasic® is a combination of different active ingredients combining, more specifically, naturally occurring Biotrinine and ATP.
- Triphasic® combats progressive hair loss in men and women; three months of treatment led to a significant improvement in hair thickness, objectively assessed by phototrichogram in men and self-assessed in subjects of both sexes. The treatment led to an overall improvement in subjects of both sexes, as assessed by the investigator.

Support

This study was supported by René Furterer.

Conflicts of interest : occasional interventions for René Furterer.

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