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A NOVEL HERBAL FORMULATION ENHANCING PROTEIN SYNTHESIS SPECIFIC FOR HAIR GROWTH - A NORTHERN BLOT ANALYSIS

JR. Krishanamoorthy[1], S. Ranganathan[2], S. Gokulshankar[2], M.S. Ranjith[2]
[1]Dr JRK Sidda Research and Pharmaceuticals (P) Ltd., Kuntrathur, Chennai - INDIA
[2]Department of Microbiology, The New College, Chennai - INDIA

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Summary

The hair growth enhancing property of a herbal oil (Keshraksha oil) and herbal hair vitalizing cream (Keshraksha cream) was studied. The total hair count assay in the guinea pig model revealed that both the oil and the cream has a definite role in increasing the hair growth. To confirm the possible mechanism of action of the formulations on the hair growth, northern blotting was performed using the Shh gene. An increased m RNA synthesis was recorded in the dermal papillae cells of the hair of the animal treated either with oil or cream but not in the control. The present study suggests that the oil and cream treatment enhances protein synthesis in the matrix cells, thereby increasing the anagen phase of the hair.

Riassunto

E' stata controllata l'attività stimolante sulla ricrescita dei peli di un olio estrattivo di origine vegetale (olio di keshrasha) e di una crema rivitalizzante prodotta con lo stesso derivato.
Il conteggio totale dei peli di un porcellino d'India, utilizzato come modello, hanno posto in evidenza come entrambi i prodotti svolgano un ruolo interessante sulla ricrescita dei peli. Per confermare il possibile meccanismo di azione dei due prodotti è stata utilizzata una specifica metodica basata sull'uso del gene Shh.
È' stata così riconosciuta una incrementata sintesi del m RNA a livello delle cellule della papilla dermica dei peli degli animali trattati sia con l'olio che con la crema, mentre tale incremento non si è verificato nel controllo.
Lo studio suggerisce che sia l'olio che la crema sembrano essere in grado di incrementare la sintesi proteica a livello delle cellule della matrice, incrementando la fase anagen del pelo.

INTRODUCTION

Hair follicles are the anatomic "factories" that have as their principal function the production of hair. Hair follicle neogenesis occurs in the embryo by invagination of the epidermal placode into the surrounding dermis [1-4]. Postnatal follicles undergo a cycle of renewal in 3 phases: anagen (growth), catagen (regression), and telogen (resting) [5,6]. The first complete postnatal hair follicle cycle (first anagen, first catagen and first telogen) is completed in the first 3 1/2 weeks after birth and is followed by the second hair cycle (second anagen, second catagen and second telogen).

Hair forms the major cosmetic apparatus of man. Hair care has been in practice since antiquity. Even in the 14-16 century English literature, the importance of the hair in the cosmetic aspect of man has been well documented (Queen Elizabeth- W Shakespeare).

Similarly, the ancient Tamil literature too contains several mentions of hair care [7].

Hair loss and premature graying of hair especially in the early and mid age group are the major cosmetic concern that plague mankind all over the world. Use of wigs and hair re-plantation although available, as an alternative is not in wide use due to the exorbitant cost and clear distinctivity. As on date, no formulation effectively addressing a solution to this problem is available.

In the present paper we have studied the effect of Keshraksha oil and Keshraksha Hair vitalizing cream*, formulated based on the aqueous and or oil extracts of certain plants viz., Phyllanthus embelica, Bergera koenigit, Lawsonia alba, Indigofera tinctoria, Wrightia tinctoria, Hibiscus rosasinensis, Muraya paniculata, Terminalia chembula and black catachu on hair growth in Guinea pig model. All the above plants are well documented in the texts of Indian system of Medicine (Siddha and Ayurveda) for enhancing the hair growth [8,9].

The role of the Keshraksha oil and cream on hair growth in Guinea pigs was studied by total hair count technique. Further the mechanism of action of the above formulations on hair growth was studied by determining the expression of mRNA specific for hair growth using Sonic Hedge Hog Gene (Shh cDNA) as probe. Further, the expression of mRNA specific for tyrosinase, an enzyme essential for melanogenesis was also studied by Northern blotting.

MATERIALS AND METHODS

Preparation of Keshraksha oil and Keshraksha hair vitalizing cream

Oil extracts of Phyllanthus embelica, Bergera koenigit, Lawsonia alba, Indigofera tinctoria, Wrightia tinctoria and Hibiscus rosasinensis were prepared using Coconut oil and adjusted to a concentration of 250 μg of each of the plant extracts in 1 ml of oil using standard procedure (10-11). So prepared oil was referred as Keshraksha hair oil.

Similarly the aqueous extracts of Lawasonia.alba, Muraya paniculata, Wrightia tinctoria, Terminalia chembula and black catachu were prepared and incorporated into hair cream base at a concentration of 100μg of each of the extract in 1g of the cream. So prepared cream was referred as Keshraksha hair vitalizing cream.

Studying the effect of oil and cream on hair growth in Guinea pigs by total count technique

A total of 4 Guinea pigs were divided into 2 groups of 2 animals each. One group was used to study the effect of Keshraksha oil on hair growth while the other was used for studying

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the effect of cream. Left and right sides of the flank region in all the animals were tonsured in both the groups and 2-cm diameter zone was marked using a permanent marker pen. In one group, the oil was topically applied over the tonsured flank region in the right side, while the left side of the tonsured flank region in the same animal was left free without any applicant (control). Similarly, in other two animals, the cream was applied in the right side of the tonsured flank region, while the left side of the flank region was maintained as control without any applicant. The treatment procedure was continued for a period of 9 days with single application per day. The total number of growing hairs in the demarcated (2 cm diameter) zones treated either with oil or cream was counted separately using a magnifying hand lens at regular time intervals (3,6,9 days) and was compared with the control [12].

RNA QUANTIFICATION

The cells of the dermal papilla from the treated (cream and oil separately) and control hair were collected and the RNA was isolated. The quantification of the total RNA isolated was done spectrophotometrically at 260nm using standard procedure [13].

Northern blotting

Northern blotting was performed with the RNA isolated from the matrix cells (cream treated, oil treated and control separately) on the 9th day using standard procedure [14]. The isolated RNA (10mg/lane) was separated on a 1% agarose gel, transferred to a nylon membrane and hybridized with [^{33}P]-labeled Shh cDNA probe. Equal loading of RNA was confirmed by analysis of GAPDH mRNA. Similarly the tyrosinase gene expression was also studied [15,16]. The hybridized mRNA was subjected to autoradiography.

RESULTS

Effect of oil and cream on hair growth by total hair count technique

A significant increase in the total number of hair grown in either cream or oil treated areas in the flank region of Guinea pigs was recorded from 3 day of treatment through 9 days, but not in the control (Table-I).

Similarly, the length of the hair was also recorded to be long in cream or oil treated areas than the control (Table-II).

Northern blotting

The mRNA expression specific to Shh cDNA was recorded in the matrix cells isolated from either oil or cream treated hairs but not in the control. The molecular weight of the transcript was recorded to be 3.1 kb corresponding to the probe length (Fig 1 and Fig 2).

Similarly, gene expression specific to tyrosinase enzyme was recorded in the matrix cells of the treated hair, but not in the control (data not shown).

Table I

Effect of cream and oil treatment on hair growth by total hair count technique

Treatment group	No. of animals	Total No. of hair grown at Different time intervals (days)					
		3		6		9	
		Mean	SD	Mean	SD	Mean	SD
Cream	2	170	0,72	280	0,28	212	0,57
Oil	2	190	0,83	264	0,51	279	0,18
Control	4	87	1.34	91	1.23	107	1.83

Table II

Effect of cream and oil treatment on hair growth – determination of length of the hair (mm)

Treatment group	No. of animals	Mean length of the hair grown at different time intervals (days)		
		3	6	9
Cream	2	+	++	+++
Oil	2	++	+++	+++
Control	4	-	+	++

- \- Negligible hair length
- \+ Hair length up to 2mm
- ++ Hair length 2 to 4 mm
- +++ Hair length 4 to 6 mm

DISCUSSION

Hair follicle is one among the human tissues containing stem cells [17]. The stem cells are interspersed within the basal layer of the outer root sheet and also in an area called bulge. From these reservoirs, the stem cells migrate to the hair matrix and then divide and differentiate to hair [18]. The division of the hair matrix is controlled by numerous cytokiues produced by the cells of the dermal papilla [12]. The hair fall resulting in baldness is mainly due to the androgens, which influence the synthesis and release of cytokines by the dermal papilla cells [19-21]. The production of androgen as a single entity largely affects the hair growth than any other physiological process in a mammalian system. The cytoplasm and nucleus of the dermal papilla cells have abundant collection of androgen receptors.

An increased anagen phase requires an effective involvement of both matrix and dermal papilla cells. The basic fibroblast growth factor (bfGF) and platelet-derived growth factor (PDGF) are known to potentiate the growth of dermal papilla cells. Similarly, the insulin like growth factor I (IGF-I) is known to accelerate the hair growth in a concentration dependent manner [22-24].

The action of IGF-I is modulated by proteins (insulin like growth factor binding protein – IGFBPs) produced by dermal papilla cells.

Northern blot analysis of mRNA specific to Shh cDNA in treated and control animals

Fig.1 **Fig.2**

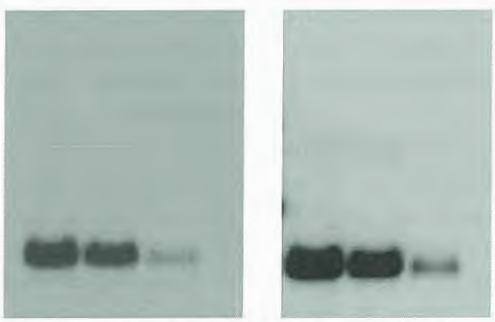

Fig. 1 *mRNA expression patterns specific to Shh cDNA in the hair follicle cells (matrix) collected from oil treated and control area.*
Lane 1 and 2 are the mRNA expression pattern of oil treated hair.
Lane 3 Untreated (control).

Fig. 2 *mRNA expression patterns specific to Shh cDNA in the hair follicle cells (matrix) collected from cream treated and control area.*
Lane 1a and 2a are the RNA expression pattern of cream treated hair.
Lane 3a Untreated (control).

However, the exact mechanism of modulation is not yet understood [25]. It has been shown that IGFBP3 (which is the most abundant type present in the dermal papilla cells) forms a complex with free IGF-I (available for the stimulation of hair elongation and maintenance of the anagen phase) and thereby influences the hair growth.

Animal studies have shown that substance P induces transition of hair from telogen to anagen phase [26]. The same effect has been observed with the active principle of chilli and pepper, which release substance P from the nerve endings in the skin. The snbstances P also known to bind to receptor on C-type afferent nerve fibers and produce pain [26].

The currently available drugs for hair growth can be broadly classified into 3 categories viz., cytotoxic drugs, anti-androgens and drugs acting on potassium channels. Anti-androgen drugs, either reduce the androgen hormones or block the transfer of testosterone to 5-DHT or block the androgen receptors in the dermal papilla cells, have met with severe consequences due to various side effects.

The action of cyclosporine, minoxidil and diazoxide on hair growth is by opening the potassium channel in the cell membrane leading to hypertrihotic action [27].

The total hair connt assay has revealed that the topical application of the Keshraksha oil and Keshraksha hair vitalizing cream has a significant effect on hair growth in Guinea pig model. Interestingly, the length of the hair in the oil and cream treated areas was also found to be longer than the hair in the untreated (control) area during the study period. Although the observation we made in the animal model could not be directly extrapolated to humans, where the hair growth in humans is in a mosaic pattern instead of the wave pattern as seen in animals [16]. However, we have recorded an increased RNA synthesis in the matrix cells of either cream or oil treated hair but not in the control. In the light of the increased hair growth recorded in the oil and cream treated region coupled with increased RNA synthesis appears significant.

We have studied the possible mechanism of action of oil and cream formulations on hair growth by northern blot analysis. For this purpose, we used the Sonic Hedge Hog gene (Shh cDNA) as probe to determine the specific mRNA expression. Shh is one of the well-known and well-studied genes responsible for hair growth in both pre-natal and post- natal skin [16,28,29].

In our study, we have recorded an increased mRNA expression specific to Shh cDNA in the matrix cells treated either with cream or oil but not in the control. The molecular weight of the transcript was recorded to be 3.1 kb, which was comparable to the length of probe. The above finding suggests that the oil and cream treatment may enhance the protein synthesis essential for hair growth. However it is not clear, whether, the ingredients in the oil and cream can also increase the production of various cytokines by dermal papilla and other mechanisms involved in the hair growth. Interestingly, mRNA expression specific to tyrosinase gene was also recorded in the matrix cells treated eitber with cream or oil but not in the control. It is already known that, tyrosinase enzyme catalyze the amino acid tyrosine, which is transformed through dopa to dopaquinon, an essential biochemical product in the melanin synthesis.

We have already established that the oil extract of Wrightia tinctoria and Muraya paniculata can enhance the phagocytosis and phagocytic killing of an antigen by the peritoneal macrophages (data communicated for publication). The uptake of synthesized melanin by the matrix cells are through the process of phagocytosis. Therefore we presume that, the application of cream and or oil besides enhancing the anagen phase of the hair, can also enhance the melanogenesis

and uptake of melanin by the hair.

At present we are evaluating the hair growth enhancing property of cream and oil formulations in humans. Nevertheless, the findings of the present study suggest that an herbal solution to baldness is in near future.

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Author Address:

Dr. S. Ranganathan
No.21 (Old No.6),
Sivaji Street, T. Nagar,
Chennai-600 017 - INDIA
Phone: 044 4338982.
Email: drsranganathan@yahoo.com