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SATURA ROSTA PREPARATION RESTORES HAIR GROWTH AND PROMOTES ADIPOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS

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Abstract

The preparation "Satura® Rosta" (Satura) has for many years been known as an effective medication for baldness treatment. Nevertheless the mechanisms of its actions are unknown. To investigate it, we performed the histological examination of scalps of two volunteers having completed a full Satura treatment. This study revealed substantial restoration of hair growth, augmentation of subcutis and an overall skin structure normalization. The existing reduced nonfunctional follicles enlarged and began to work. In addition to the histological examination, we found that Satura induced adipogenic differentiation in the cultures of mouse stromal cells from bone marrow. As far as subcutis, it is the normal environment for anagen hair follicles and adipogenic differentiation can be the prerequisite of hair growth. On the other hand alopecia and the decreasing of subcutis are the main manifestations of skin aging. Various aspects of the possible mechanisms of Satura action are discussed.

Keywords: alopecia, Satura, adipogenic, stem cells, hair growth, human, mice

INTRODUCTION

There are many kinds of hair loss (alopecias) that differs in the degree of manifestations and mechanisms for development. Among the etiological factors highlighted were congenital or genetic disorders, autoimmune diseases, manifestations of senescence or premature aging, consequences of anticancer therapy or traumas and even psychological stress. Hair loss occurring throughout life can be alternatively divided into inflammatory and non inflammatory types. Pathogenesis and etiology of different types of alopecias are not completely understood therefore for hair growth reconstruction the only current remedy is to apply numerous different types of treatments most of which are either not very effective and or have essential drawbacks.

Most widespread type of alopecia (more than 90%) are androgenetic (AGA). It is characterized in men by the appearance of focal zones of baldness in frontal and parietal areas and in women it is characterized by hair rarefication in the same areas. Repeated hair cycles with shortened anagen phase leads to vellus transformation of scalp hairs and correspondent hair follicle miniaturization. All the hairs in an affected area may be involved in the miniaturization process. Along with hair miniaturization the production of pigment ceases. AGA is a very actual problem because 50% of men beyond the age of 50 years and 50% women beyond the age of 60 years are subject to AGA (1). Although AGA is a benign medical condition, affected individuals experience great psycho-emotional stress, often leading to a reduction of quality of life.

The mechanisms of AGA are relatively well investigated. From the early forties it is known from clinical evidences that the process is mediated mainly by androgen (2). Briefly this disease is characterized by increased responsiveness of some dermal papilla cells (in specific locations) to testosterone that leads them to increased secretion of cytokines like TGF-β and DKK-1 that inhibit hair growth. TGF-β (3-5) is known as a catagen inducer in hair cycling, DKK-1 (6) inhibits the growth of the outer root sheath cells and triggered apoptotic cell death. It is likely that these paracrine mediators determine an androgen-induced suppression of hair growth and is an early catagen induction in AGA (7).

For many years "Satura® Rosta" (Satura) has been known as an effective remedy for baldness treatment, but the mechanisms of its action are unknown. Presented investigation shows that Satura encourages whole skin regeneration including restoration of hair growth and the augmentation of subcutis. We discovered that

Satura promotes adipogenic differentiation of the mesenchymal stem cells. This effect can explain the hair growth promoting action of Satura. Further experiments are in this schedule to explore Satura's properties.

MATERIALS AND METHODS

Satura preparation

"Satura® Rosta" (Manufactured by Propico Inc., Moscow) is a preparation for baldness treatment. It has been used since 1989 in various countries (USA, Great Britain, Israel, and Russia). This preparation has been tested many times in different laboratories to comply with the safety requirements and sanitary regulations for perfumes and cosmetics. No microorganisms are revealed. Toxic properties comply with the standards and it provides no skin-irritant or allergenic effects (8-11). The exact composition (Intellectual Property and Methodology) of Satura is wholly owned and trade marked by Propico Inc. The General Director of Propico Inc. Dr. G.V. Zigmond has advised the authors that the basis of Satura is the processed mixture of numerous plant and seaweed extracts.

We used "Satura® Rosta" (Satura) for human investigations. For experiments with cell culture we used the basis of the balsam that constitutes only 5% of the balsam. This basis was provided by Dr. G.V. Zigmond. We prepared a 1% ethyl alcohol solution and typically used 0,001% final concentration.

Clinical Studies

Two patients (male) with androgenetic alopecia who previously undertook medical advice were selected for biopsy investigations. They both represented typical cases of alopecia, were 40 and 45 years old and had no any other pathologys (were apparently healthy). Written informed consent was obtained from them. Biopsies were taken from each in a cosmetic surgery clinic from the same affected areas of scalp before and after four months of a conventional Satura treatment.

Histology

The obtained skin samples were fixed with formalin, embedded in paraffin and then the sections were prepared and stained with hematoxylin-eosin or according to Van-Gieson (12).

Cell culture

Cultures of mice bone-marrow derived mesenchymal stem cells (mMSC) were obtained from C3H mice as described (13). Cells were grown in DMEM medium (PanEco, Russia) supplemented with 10% fetal bovine serum (FBS) (HyClone, USA), 2mM L-glutamine (PanEco, Russia) and 40 μg/ml gentamycin (PanEco, Russia) at 37°C in the presence of 3% oxygen and 5% CO_2. The culturing of cells under low oxygen conditions was performed in a special incubator (model 484, ThermoForma, USA). For adipogenic assay cells were incubated in adipogenic medium (DMEM with addition of 10% FBS, 0.5 mM isobutyl-methylxanthine, 1 μM dexamethasone, 10 μM insulin, 200 μM indomethacin) (14). For lipid detection, the fixed cells were stained for 10 min with a filtered solution of Sudan 4. The stained cells were then briefly rinsed three times with water then photographed. Photography of the cells and slides were carried out on a Nikon Diavert inverted phase-contrast photomicroscope by using a Nikon D5000 camera. Images were contrasted by using Image J v1.23 software (Scion).

RESULTS AND DISCUSSION

During the course of the Satura treatments of the two cases of androgenetic alopecia, along with regular medical examinations, we carried out histological investigations of microscopic scalp sections. Direct observation of the scalp before the treatment revealed very rare density of long hair that increased substantially by the end of treatment four months later. The patients were satisfied with the results obtained.

Microscopic observation of the skin before treatment demonstrated that the main component of dermis was sebaceous glands without hair shafts. The structure of the dermis was irregular with occurrence of empty spaces. Some follicles are degenerated. Subcutis is feebly marked (fig. 1). There are damaged and catagen hairs.

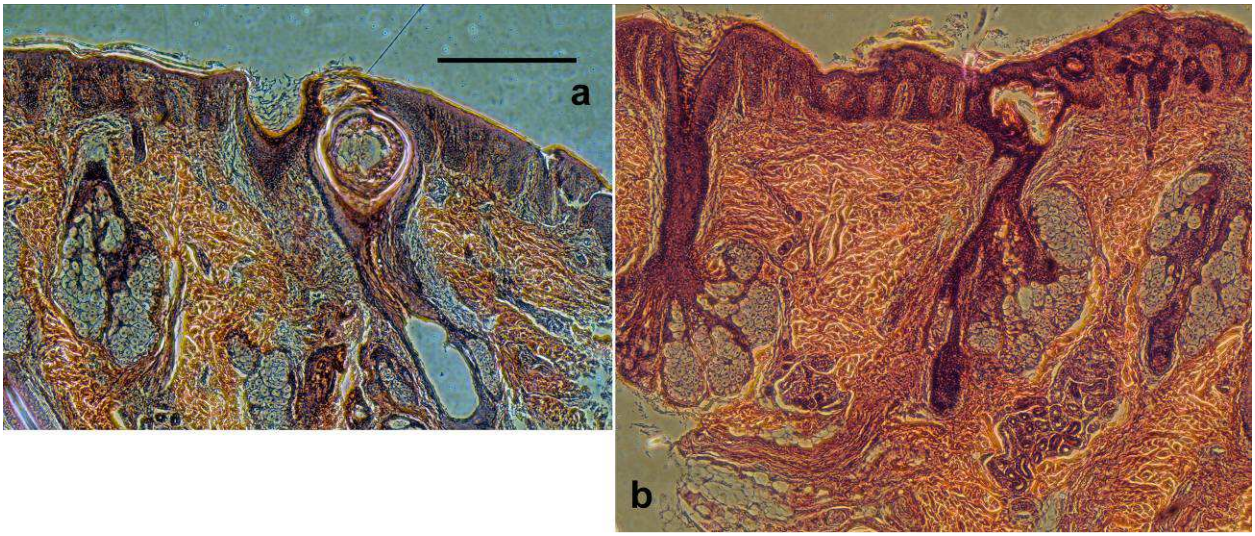

Fig. 1. Scalps before Satura treatment. a. - patient #1, b - patient #2. Hematoxylin-eosin staining. Phase contrast, digital contrast. Bars=500µ.

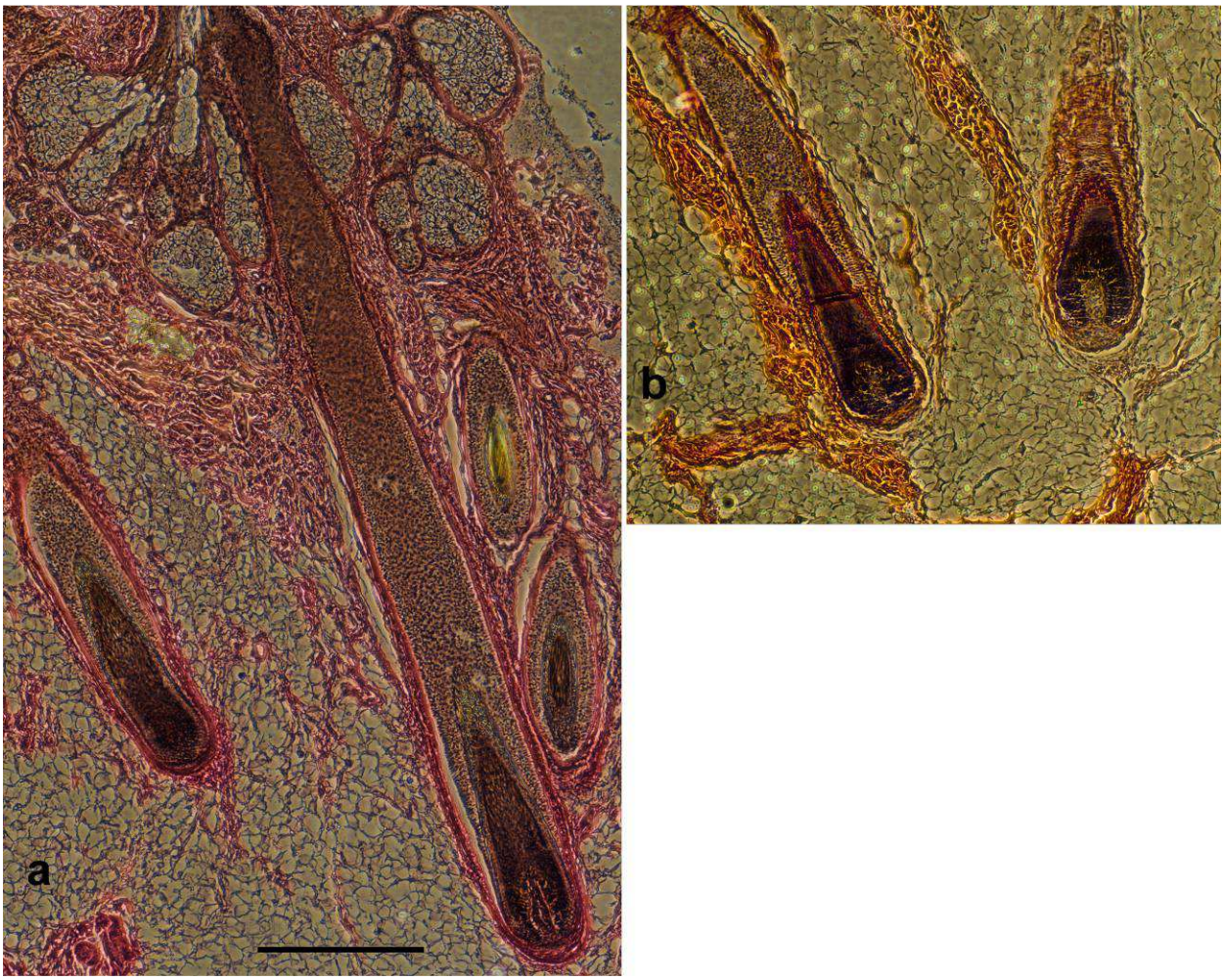

Fig. 2. Scalps after 4 months of Satura treatment. a. - patient #1, b - patient #2. a – Van Gieson staining, b - hematoxylin-eosin staining. Phase contrast, digital contrast. Bars=500µ.

At the end of four months, Satura treatment histological analysis revealed an increased quantity of anagen hairs (fig.2). The skin increased their depth mainly for account of subcutis enlargement. The skin structure became more regular. Most of the follicles had increased size. They had pronounced dermal papilla (Fig. 3b).

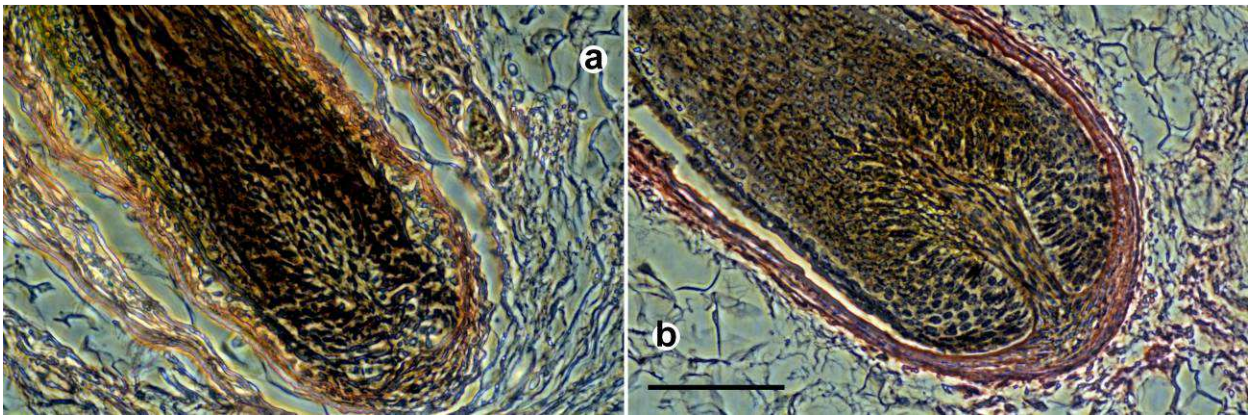

Fig. 3. Hair follicles before and after Satura treatment. Patient #1. a - before; b – after treatment. Van Gieson staining. Phase contrast, digital contrast. Bar = 100µ.

Thereby we satisfied oneself that Satura treatment significantly improve the condition of two patients with AGA. That improvement was confirmed with histological examination. To take into account previous long experience of this preparation in the baldness treatment it become clear that its efficiency (at least in these two cases) is on the level of the best preparations using for AGA treatment. As in the case of minoxidil treatment hair growth appears to peak approximately four months after initiation of therapy (15). As opposed to minoxidil the effects of the Satura treatment are much more prolonged, although in some very difficult cases a small number of patients may need a repeated course of treatment after few years. (Information supplied by Propico Inc.)

A histological analysis of the human scalp after the Satura treatment reveal, first of all, the increase of hair follicles sizes of up to 200µ in diameter and even more (fig.3). This effect is the reverse motion relating to AGA when progressive hair follicle miniaturization takes place. Corresponding to follicles increased growth one can see expansion of subcutis that becomes very spongy (compare fig. 3a and b). All other components, first of all blood vessels also elaborate. All these processes can be estimated as whole skin regeneration that resembles rejuvenation.

As the development of subcutis is the prerequisite of hair growth we checked the ability of Satura to influence on the differentiation of stem cells. We observed that Satura indeed induces an adipogenic differentiation of mice MSC (Fig. 4). The effect was weaker that from standard differentiation protocol in respect of ratio of differentiated cells, but it significantly differed from the control cells where we could not observe spontaneous adipogenic differentiation.

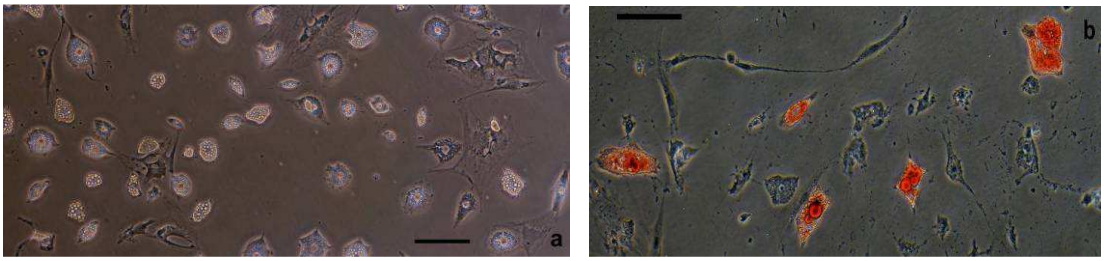

Fig. 4. Satura induces adipogenic differentiation of mouse bone marrow stem cells. a - phase contrast, b – phase contrast and Sudan 4 staining. Digital contrast. Bars=100µ.

The stimulation of adipogenic differentiation by Satura can be evaluated as a step directed to the elaboration of subcutis in the process of skin rearrangement (16). Modern science considers adipose tissue transplantation as a tool for regenerative medicine (17). It is known that subcutaneous fat transplantation has a profound effect on metabolism (18). Adipose tissue actively participates in homeostasis by secreting numerous cytokines (19). Among secreting by adipose tissue cytokines the following factors are noted: epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), transforming growth factor-beta (TGF-β), insulin-like growth factor (IGF), brain-derived neurotrophic factor (BDNF), Flt-3 ligand, granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-11 (IL-11), interleukin-12 (IL-12), leukemia inhibitory factor (LIF), and tumor necrosis factor-alpha (TNF-α) (20).

Adipose tissue secretes angiogenic and anti-apoptotic growth factors in sufficiently high bioactive levels (21). It is likely that HGF is the central angiogenic factor secreted by adipose tissue. Its suppression has been shown to inhibit the angiogenic and regenerative effects (22). Besides angiogenic and anti-apoptotic factors, adipose tissue secretes virtually all of the cytokines that take part in normal wound healing (21, 23). Kim et al. (2007) demonstrated that adipose tissue stimulates not only skin fibroblast proliferation and migration but also secretion of type I collagen. Adipose tissue promotes wound healing by increasing the vessel density, granulation tissue thickness, and collagen deposition (24), and they also improve the cosmetic appearance of any resultant scars (25, 26).

So, the stimulation of adipogenic differentiation can lead to subcutis elaboration and intensification of hair growth by providing sufficient cytokines, energy, and space. From the practical usage of Satura it is known that it possesses a pronounced anti-inflammation activity that easily reveals after burns treatment (Propico Inc. information). It is likely that induction of adipogenic differentiation and anti-inflammation activity are interlinked. It is shown that adipose tissue possess some antioxidant action (27). From the other hand it is known that various anti-inflammation agents such as dexametasone, indometacine possess adipogenic activity (28, 29).

Therefore having ascertained the ability of Satura to have a long lasted effect it is possible to assume that Satura enlarges follicle sizes to such an extent that the whole case began to resemble the beginning of alopecia development. It is known that baldness appears with long delay after testosterone increasing. In these circumstances hair growth continues without being apparently affected by the persisting toxic influences of dihydrotestosterone that previously had lead to AGA.

Since Satura is very complex mixture of plants derivatives it is likely that its action in vivo is not restricted by adipogenic stimulation. It is possible that Satura activates innate immunity, autophagy, has anti-inflammation and anti-aging action etc. This preparation is needed in further investigations.

CONCLUSIONS

We established that Satura preparation restores hair growth and promotes whole skin development including the elaboration of subcutis and the enlargement of hair follicles. Its action in the early stages (after a few months) resembles the most effective preparation against alopecia. In contrast to all other preparations, the effect of Satura is prolonged and indicates the existence of particular mechanisms of its action that differ from all others. It is probably, these mechanisms include the induction of adipogenic differentiation that leads to subcutis development which is a prerequisite of the anagen stage of hair growth.

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