Efficacy of a Hair Tonic Containing Human Umbilical Cord Blood Mesenchymal Stem Cell-derived Conditioned Media in Patients with Androgenetic Alopecia

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Background: The development of a safe and convenient agent that can promote hair growth in patients with androgenetic alopecia remains challenging.

Objective: This study was designed to investigate the efficacy of a newly developed hair tonic containing a human umbilical cord blood mesenchymal stem cell (hUCB-MSC)-derived conditioned medium in promoting hair growth.

Methods: This double-blind, placebo-controlled clinical study investigated the efficacy of a hair tonic containing an hUCB-MSC-derived conditioned medium in 30 patients with patterned hair loss. Treatment efficacy was determined using phototrichograms to evaluate the density, diameter, and hair growth rate at baseline levels and after 4, 8, and 16 weeks of treatment.

Results: The hair density in the group treated with the hair tonic significantly increased from 125.2 to 134.6 hairs/cm² (p < 0.05). In this same group, the thickness of hair also increased from 0.083 to 0.110 mm (p < 0.05). Additionally, the hair growth rate increased from 0.285 to 0.338 mm/day (p < 0.05). No severe adverse reactions were reported.

Conclusion: A hair tonic containing an hUCB-MSC-derived conditioned medium could be a new effective alternative to treat patients with androgenetic alopecia. (Korean J Dermatol 2019;57(5):251～257)

Key Words: Androgenetic alopecia, Hair preparations, Mesenchymal stem cells, Umbilical cord

INTRODUCTION

Androgenetic alopecia is a common form of hair loss in both men and women. Androgenetic alopecia affects approximately 50 million men and 30 million women in the United States. It can begin to manifest as early as the teenage years and shows increasing incidence with age. In fact, >50% of men aged >50 years show some degree of hair loss. Hair loss in women is most likely to occur after menopause. Thus, researchers have shown significant interest in developing a safe and effective treatment option to promote hair regrowth. Additionally, an effective solution to androgenetic alopecia is expected to have a high market value.

Recent research aimed at treatment of androgenetic alopecia has focused on the hair follicle as the primary source of multipotent stem cells. Umbilical cord blood (UCB) is a useful source of mesenchymal stem cells (MSCs) owing to its abundant availability and ease of collection. A few studies have reported that primitive UCB-MSCs demonstrate biological advantages over bone marrow- or adipose tissue-derived MSCs (ADSCs), suggesting that UCB-MSCs may serve as a useful model for the clinical application of cell therapy. For example, UCB-MSCs cultured with specific substances produce growth and regulatory factors that show paracrine effects on surrounding cells. These growth factors, which include vascular endothelial growth factor (VEGF), hepatocyte growth factor, insulin-like growth factor, and platelet-derived growth factor, have also been shown to affect hair growth by promoting angiogenesis, maintaining
anagen hair, and activating hair growth. Previous studies have reported the development of dermal papilla-like tissue from UCB-MSCs following exposure to a dermal papilla-forming medium. These reports suggest that human UCB-MSCs (hUCB-MSCs) may produce a therapeutic effect in patients with hair loss, and several previous studies have reported that conditioned media obtained from ADSCs promote hair growth in vitro and vivo.

In this study, we evaluated the efficacy and safety of a hair tonic containing an hUCB-MSC-derived conditioned medium in 30 patients with androgenetic alopecia.

**MATERIALS AND METHODS**

1. **Subjects**

This study included physically and mentally healthy adults aged 20~60 with a diagnosis of mild-to-moderate patterned hair loss (men: modified Norwood-Hamilton classification types II, Ila, Ilv, IIIa, or IIIv and women: Ludwig classification Type I). Patients with a history of any skin or scalp disorders, endocrine abnormalities, or systemic diseases associated with liver dysfunction were excluded from this study. To prevent the effect of other treatment on our results, we excluded patients who had previously received other treatments for androgenetic alopecia, including those who received finasteride, topical hair restoration agents, or surgical treatments such as hair transplants and scalp reduction. Pregnant and nursing women were also excluded. This study was reviewed and approved by the Institutional Review Board of the P&K Skin Research Center (IRB No. P1509-46) and was performed in accordance with the principles of the Declaration of Helsinki, Korean Good Clinical Practice guidelines, and local regulatory requirements. All patients provided informed consent prior to study participation.

2. **Isolation and culture of human umbilical cord blood mesenchymal stem cells**

Mononuclear cells derived from cord blood were isolated by centrifugation and were washed several times to remove any remaining foreign bodies. These cells were cultured as a monolayer at an appropriate density. The cultured spindle-shaped mesenchymal cells formed homogeneous colonies, and these cells were allowed to proliferate until a sufficient number of cells could be acquired during passaging.

3. **Preparation of a stimulated conditioned medium**

Cells previously stored in liquid nitrogen were defrosted and cultured at 37°C in a 5% carbon dioxide incubator. The cells were grown in α-Minimum Essential Medium (α-MEM, GibcoTM, Carlsbad, CA) supplemented with fetal bovine serum (FBS, GibcoTM) until they reached 90% confluency. To collect the conditioned medium, the cells were washed thrice with phosphate-buffered saline (PBS), and the stem cells were stimulated in a phenol red-free medium supplemented with 10 ng/mL of transforming growth factor-beta (TGF-β) and lithium chloride (LiCl). The medium was collected 24 hours later, and the cells were again washed thrice with PBS, prior to the addition of the keratinocyte-serum free medium (K-SFM, GibcoTM). We collected the culture medium every 24 hours for 3 days. The medium was filtered, TGF-β and LiCl were added, and the resulting solution was used at a 10% dilution.

4. **Study protocol**

This double-blind, placebo-controlled clinical trial included patients who were categorized using stratified permuted block randomization, the randomization table for which was generated by a statistician using an allocation code. A hair tonic containing either the hUCB-MSC-derived conditioned medium or a placebo was distributed to patients (1:1 ratio). Patients from both groups were instructed to apply the tonic twice daily for 16 weeks (day and night, 1 mL/dose) and massage the scalp for 1~2 min during application. The hair tonic used in this study contained a 10% solution of hUCB-MSC-derived conditioned medium treated with LiCl and TGF-β. The placebo tonic contained alcohol without any conditioned medium, and this placebo was to be applied by patients from the control group in a manner similar to the test group.

5. **Assessment**

Prior to performing hair measurements, patients with hair existing in the area affected by patterned hair loss were instructed to shave a circular area measuring 1 cm in diameter to a length <2 mm near the crown of the head. The target area at the center of the circle was marked with a 1-mm black dot (tattoo). Hair density and diameter were objectively assessed by a phototrichogram (Folliscope 4.0®, Lead M Co., Seoul, Korea) before using the hair tonic (baseline measurement) and were re-measured after 4, 8, and 16 weeks of treatment. Hair density (hair count/cm²) was calculated by counting the total number of hairs in the target area. Hair thickness (mm) was calculated using the mean diameter of 5 hairs measured manually in the target area. The following formula was used to determine the rate of hair growth: hair growth rate (mm/day)= (hair length the number of days after shaving minus hair length immediately