Histological Study on the Effect of 1550 nm Erbium Glass Fractional Laser Treatment of Androgenic Alopecia

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Abstract

Androgenic alopecia (AGA) is a non-scarring hair loss problem caused by multiple etiologies, including genetic, hormone androgen, and micro-inflammation of the perifollicular area. The standard treatment for AGA is topical minoxidil and systemic 5α-reductase inhibitor which is still limited by its efficacy and side effects. A 1550-nm erbium glass laser is one of the effective treatment modalities for AGA that show a satisfactory result. However, the mechanism of how this laser stimulates hair regrowth is still unclear. Macrophages have been identified as an important role in hair cycling. The authors histologically evaluated changes in macrophage expression in balding scalp areas of subjects with an improvement, comparing pre and post-laser treatment to identify the mechanism of laser-induced hair growth. A total of 12 samples were stained with CD68 and evaluated by an experienced pathologist. Nonetheless, the authors failed to demonstrate any of these alterations. In conclusion, to diminish the negative finding, it is suggested to perform optimal laser parameters and evaluate the right tissue plane.

Keywords**:** *hair loss, fractional laser, macrophages, wound, inflammation*

1. Introduction

Androgenic alopecia (AGA) is one of a non-scarring hair loss disorders which has been known as the most common type of hair loss problems in both male and female (Meephansan et al., 2018). AGA has a major impact on individual self-esteem through psychological health. It is widely believed to be an androgen-dependent, genetically determined trait, and is characterized by a progressively shortened anagen, prolonged telogen, and miniaturization of the scalp hair follicle (Meephansan et al., 2018). Males generally present with the recession of hairline at the temples and vertex balding, while females are typically diffusely thin over the crown area with preserving the frontal hairline (McElwee and Shapiro, 2012). In Thailand, the prevalence of male pattern hair loss is about 38.52% (Pathomvanich et al., 2002), and increased with age (Lee and Lee, 2012). The standard treatment of the AGA is topical minoxidil and systemic 5α-reductase inhibitor which is still limited by its efficacy and side effects (Rogers and Avram, 2008).

Extensive studies have recently been conducted to search for an effective AGA treatment strategy, including utilizing a fractional laser. Recognition of laser mechanisms will be beneficial in AGA treatment modalities. A 1550-nm erbium glass (Er:Glass) fractional laser is one of the new effective treatment options that show a maximum therapeutic effect with fewer side effects for AGA (Lee, Lee, and Kim, 2011; Kim et al., 2011). Hair growth may follow the introduction of some level of wound healing (Levy et al., 2007). Some associations between wound healing and hair follicle are that the normal wound healing process has influenced hair follicles (Levy et al., 2007). Besides, many studies have been conducted on the inflammatory cells at the site of injury and infection. During wounding, monocytes are recruited and attracted to the wounded tissue 48 to 96 hours after the injury, where they are differentiated into macrophages (Zaja-Milatovic and Richmond, 2008). The macrophages in the wound bed contribute to the acute inflammation immediately after wounding and also infiltrate at the late stage of the wounding and produce various growth factors (Kasuya, Ito, and Tokura, 2018). The growth factors such as insulin-like growth factors, fibroblast

growth factors, epidermal growth factors, and hepatocyte growth factors play roles in influencing hair growth and the hair cycle in the wounding area (Lee et al., 2011). Osaka et al identified that the macrophages also play an essential role in wounding-induced hair regrowth (Osaka et al.,2007). Also, Castellana et al reported that skin-resident macrophages work as important mesenchymal regulators of epithelial hair follicle stem cell function under physiological conditions and identified a new link between the macrophages and hair follicle cycling. The macrophages turn out to be involved in hair follicle stem cell activation and anagen induction (Castellana, Paus, and Perez-Moreno, 2014). The precise functional relationship between the macrophages and hair cycling is quite poorly understood. Further studies should focus on immunocytes such as macrophages to develop a novel AGA therapeutic strategy. Interestingly, in a murine study, the parameters of 1550-nm Er:Glass fractional laser that can enhance the anagen entry should not be less than 10 mJ with 1500 MTZ/cm^2 in density. The less energy and density cannot promote reentry of anagen. The density of 1600 MTZ/cm^2 increases inflammatory cytokine expressions, including TNF-α, IL-1B, and IL-6 (Wu et al., 2015).

2. Objectives

This study aims to elucidate the mechanism of 1550-nm Er:Glass fractional laser by comparing the difference in the histology of the scalp biopsy before and after the treatment.

3. Materials and Methods

In 2017, 23 male and female patients were enrolled in our previous study, using 1550-nm Er:Glass (MOSAIC, Lutronic Co., Ltd., Seoul, South Korea) for AGA treatment with the following parameters; 2 mm×12 mm tip, 6 mJ pulse energy, 300 spot/cm^2 density, and static mode 2-4 passes at the balding area without local anesthesia for 14 sessions in a 2-week interval for 24 weeks. Scalp biopsy specimens were obtained at the baseline and 1, 2, and 3 months after the first irradiation (each group of months contained four patients). For the preparation of the histological samples, each tissue was cut transversely into 3μm, paraffin-embedded, and was stored for the examination. The authors' group reported a significant increase in hair shaft diameter and hair count since the fourth month after the treatment throughout the end of the study (Meephansan et al., 2018). However, the mechanisms of this laser-wound healing hair-regrowth remain unclear. In this present study, the authors histologically analyzed the change in macrophage expression in balding scalp areas of the subjects with improvement, comparing before and after the laser treatments to explore the mechanisms of laser-stimulated hair growth. The present study was designed as a retrospective, pilot study with a total of twelve samples. Immunohistochemical analysis was performed by staining the specimens with CD68. The specimens were then evaluated using an evaluator score which we directly compared before and after treatment slides, and scored them as positive or negative staining from the pictures. The negative result was graded as a 0 score. In the case of positive staining, the intensity of the staining was graded on a scale of 1+ to 3+. The value of each score was defined as follows; weakly positive (1+), moderate positive (2+), and highly positive (3+). Figure 1 shows different types of the frequency of cellular staining patterns scaling from 0, (1+), to (3+) as an example the authors followed during the experiment.

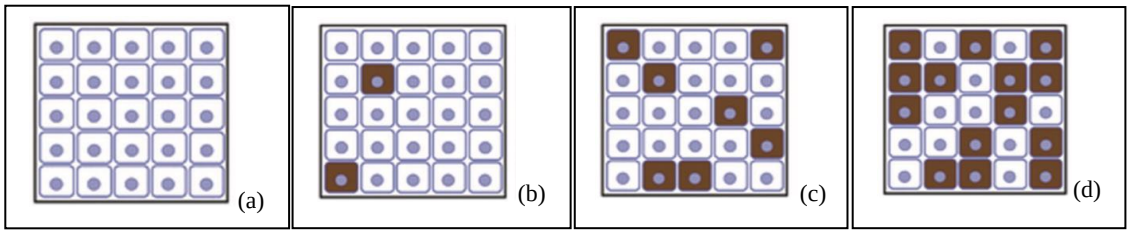

Figure 1 Types of cellular staining patterns with differences in frequency (1a) 0 score, (1b) 1+, (1c) 2+ and (1d) 3+

4. Results and Discussion

The three groups, including the baseline, first, second, and third-month groups did not show remarkable alterations. All tissues had a 0 score. Possible mechanisms for laser-wound healing hair growth included increase blood flow, induction of inflammatory cytokines and growth factors, and directed stem cell, bulge cell, or dermal papilla cell stimulation. Various molecules that play a role in wounding, including members of the fibroblast growth factor family, and interleukins, are recognized as the main factors that influence hair growth and the hair cycle (Lee et al., 2011). Importantly, macrophage infiltration upon wounding is needed for hair follicle growth since the macrophages contribute to the cyclic activation of hair follicle stem cells (Osaka et al., 2007). Though several studies have already discussed a connection between the macrophages and the regulation of hair follicle cycling (Suzuki et al., 1998; Eichmüller et al., 1998; Paus et al., 1998), this study attempted to investigate the changes in the macrophages in the balding scalp areas of the patients with improved hair growth, comparing the satisfaction pre and post-treatment to evaluate the mechanism of the laser-induced hair growth. According to literature reviews, macrophages play a fundamental role during hair follicle cycling (Paus et al., 1998; Castellana et al., 2014), which improves our understanding of the relationship between macrophages and hair growth. However, the authors failed to determine any of this alteration. Figure 2 shows that there is no infiltration of the macrophages both before and after laser irradiation. The laser parameters used in this study may not be sufficient to stimulate the inflammatory cell infiltration into the skin. Molecular changes in the wound healing response caused by thermal injury might be dependent upon laser energy. Moreover, 3μm-thick histological sections from the scalp tissues were cut horizontally, which might not be at the optimal depth as previous studies demonstrate macrophages located near the distal (close to the epidermis) of the hair follicle (Castellana et al, 2014).

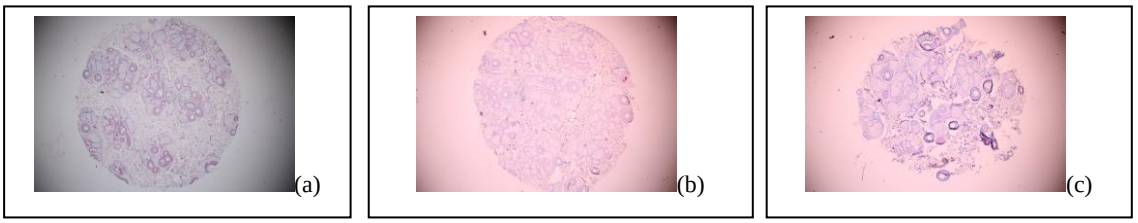

Figure 2 Infiltration of macrophages in the scalp tissues after 3 months of 1550 nm Er:Glass fractional laser (2a) baseline, (2b) 2nd month, and (2c) 3rd month

5. Conclusion

A total of 12 samples of AGA patients with an improvement were enrolled in our study. The specimens were stained with CD68 to evaluate macrophages. Since the parameters of 1550-nm Er:Glass fractional laser which can enhance the anagen entry should not be less than 10 mJ with 1500 MTZ/cm^2 in density, the laser parameters in this experiment might, thus, not be sufficient to stimulate inflammatory cell infiltration. Also, the specimens in this study were cut transversely and might not be at the optimal depth, by which the previous studies showed that the macrophages were located close to the epidermis of the hair follicle. In conclusion, to diminish these limitations, further studies using optimal laser parameters and proper histological sections should be conducted.

6. Acknowledgements

The work was supported by the staff from Chulabhorn International College of Medicine.

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