HSL Attenuates the Follicular Oxidative Stress and Enhances the Hair Growth in *ob/ob* Mice

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Summary: We demonstrated enhanced hair regeneration following topical administration of *N*-(3-oxododecanoyl)-L-homoserine lactone (HSL) in *ob/ob* mice. The *ob/ob* mice showed delayed hair regeneration (more than 6 wk) after depilation, which rapidly induced transition to anagen in the hair cycle in wild-type mice. Vehicle and HSL solutions were applied to the depilated dorsal skin of *ob/ob* mice. The depilated skin of the HSL-treated mice was fully covered with hair, whereas no macroscopic alteration was observed in vehicle-treated group by the fourth week after depilation. Oxidative stress was drastically decreased and the expression of the antioxidative enzymes PON1 and PON3 was increased in the HSL-treated skin with highly proliferative anagen follicles. These results suggest that HSL is a candidate therapeutic agent for alopecia in metabolic syndrome. *(Plast Reconstr Surg Glob Open 2013;1:e60; doi: 10.1097/GOX.0000000000000000; Published online 24 October 2013.)*

Alopecia markedly reduces a patient's quality of life[1] and is thus an important topic in nursing science. Administrations of minoxidil and finasteride are the most established treatments for androgenetic alopecia at present.[2,3] However, their efficacy varies among individuals,[4] suggesting that some intrinsic factors affect the pathology and/or treatment of androgenetic alopecia. Recently, some researchers have identified an association between alopecia and metabolic syndrome (MS).[5] We and other researchers have shown elevated oxidative stress in the skin of patients with MS[6–8] and in the scalp of patients with alopecia areata.[9] Furthermore, reduced activity of paraoxonases (PONs, antioxidant enzymes) has been shown in patients with alopecia areata.[10] Therefore, we hypothesized that the administration of the substrate of PONs would attenuate oxidative stress and enhance progression in the hair cycle in the skin of MS model mice. In this study, we demonstrated the promoting effects of *N*-(3-oxododecanoyl)-L-homoserine lactone (HSL), a substrate of PONs, applied topically to the back of *ob/ob* mice on hair growth through the attenuation of oxidative stress.

MATERIALS AND METHODS

All animal experiments were approved by the Animal Research Committee of The University of Tokyo.

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Depilation and HSL Treatment

We purchased 8-week-old male MS model (*ob/ob*) and wild-type (+/+) mice from Japan SLC (Hamamatsu, Japan). After removal of the hair from the dorsal skin using depilatory cream (day 0), 5 +/+ mice and 5 *ob/ob* mice were regularly monitored until the depilated area was mostly covered with hair.

HSL (Sigma-Aldrich, St. Louis, Mo.) was dissolved in dimethyl sulfoxide at a concentration of 10 mM and diluted to 10 μM using a 30% glycerol solution. Glycerol solution supplemented with 0.1% dimethyl sulfoxide was used as a vehicle solution. HSL or vehicle solution (50 μl) was applied to the dorsal skin of the *ob/ob* mice the day after depilation. The animals treated with HSL or vehicle (5 animals in each treatment) were regularly monitored until day 28 or killed on day 21. Skin was removed from the center of the back and the skin from each mouse was divided into 2 pieces.

Histological Analysis

One piece of tissue was fixed with 4% paraformaldehyde and embedded in paraffin. Four-micrometer-thick serial sections were stained with hematoxylin and eosin for immunofluorescence using rabbit polyclonal anti-Ki67 antibody (Novus Biologicals, Littleton, Colo.) and immunohistochemistry using mouse anti-8OHdG monoclonal antibody (JaICA, Shizuoka, Japan).

Reverse Transcription-Polymerase Chain Reaction

Total RNA was extracted from the homogenate of the other piece of harvested tissue using an RNeasy Plus mini kit (Qiagen, Venlo, the Netherlands). Reverse transcription-polymerase chain reaction was performed using a QuantiTect Reverse Transcription kit (Qiagen), AmpliTaq Gold PCR Master Mix (Life Technologies, Carlsbad, Calif.), and specific primer pairs for the following genes:

18S ribosomal RNA (*18S rRNA*): forward, TCAAGAACGAAAGTCGGAGG; reverse, CCCTTCCGTCAATTCCTTTA
Pon1: forward, TGTACCTACTGGTGGTAAAC; reverse, AAAAGCTCTCAGGTCCAATA
Pon2: forward, GTAAACCACCCACAATTCAA; reverse, CCCAGTGTAGGTTCAAGTAT
Pon3: forward, CCAAAAGAGGTCAAAGTTGT; reverse, GATCAACGGTCAAGTTATCC

The target sequences were amplified by a standard 3-step protocol: preheating at 95°C for 10 minutes, 25 (*18S rRNA*) or 40 (other genes) cycles of denaturation (95°C for 15 s), annealing (60°C for 15 s) and extension (72°C for 30 s), and a final extension step at 72°C for 1 minute. The amplicons were separated by 2% agarose gel electrophoresis (50 V, 40 min), stained with SYBR Green I, and visualized under ultraviolet light.

RESULTS

The hair-cycle phase is known to reach the second telogen phase in wild-type mice at the age of 8 weeks.[11] Depilation induced a rapid transition to anagen as shown by skin pigmentation by the first week and abundant hair regeneration by the fourth week in +/+ mice (Fig. 1A). Conversely, only slight pigmentation was observed and not until the sixth week after depilation in *ob/ob* mice (Fig. 1B).

When the HSL solution was applied to the dorsal skin of *ob/ob* mice, pigmentation was observed by the third week and the regenerated hair fully covered the depilated skin by the fourth week, whereas no pigmentation or hair regeneration was observed in vehicle-treated *ob/ob* mice (Fig. 2A). Histological examination revealed that the elongated hair follicles reached the deeper layer of subcutaneous fat tissue, the dermal papilla was completely enclosed by the developed hair bulb, and hair shaft regeneration was observed in HSL-treated skin tissue (Fig. 2B). Immunofluorescence for Ki67 revealed activated cell proliferation in the inner and outer root sheaths of HSL-treated follicles (Fig. 2C).

The level of oxidative stress was examined by immunohistochemistry for 8OHdG. A large number of follicular cells were positive for 8OHdG in the vehicle-treated *ob/ob* mice. HSL treatment drastically reduced the number of positive cells (Fig. 2D). Expression of *Pon1* mRNA was slightly higher in HSL-

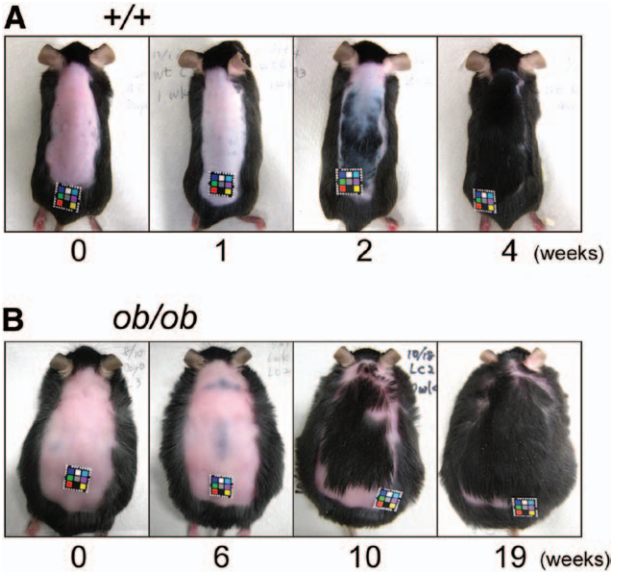

Fig. 1. Hair regeneration after depilation in 8-week-old wild-type mice (A) and *ob/ob* mice (B). Each panel shows 1 representative experiment of 5 replicates. Color chart: 1 cm^2.

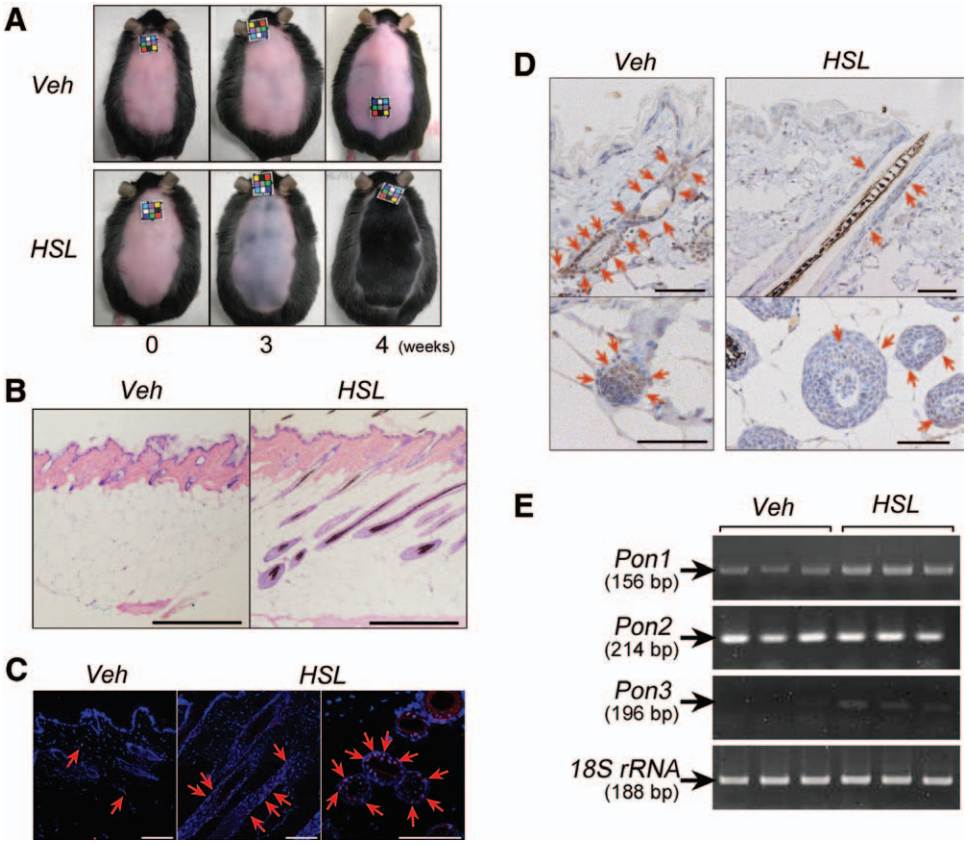

Fig. 2. Hair regeneration in vehicle-treated (Veh) and HSL-treated (HSL) *ob/ob* mice. A, Macroscopic images at 0, 3, and 4 weeks after treatment. Color chart: 1 cm^2. B, Hematoxylin and eosin staining of skin tissue sections from the center of the back of animals in the vehicle-treated and HSL-treated groups. Bar: 500 μm. C, Immunofluorescence for Ki67 (arrows), proliferative marker, with DAPI counterstaining in the vehicle-treated (left panel) and HSL-treated groups (middle sagittal plane, right horizontal plane). Bar: 100 μm. D, Immunohistochemistry for 8OHdG (arrows), oxidative stress marker, with hematoxylin counterstaining in the sagittal (upper panels) and horizontal planes (lower panels) of skin tissue in the vehicle-treated (left panels) and HSL-treated groups (right panels). Bar: 50 μm. E, mRNA expression of *Pon1*, *Pon2*, and *Pon 3* in the vehicle-treated and HSL-treated groups examined by RT-PCR. 18S rRNA was simultaneously detected as an internal control. Each panel shows 1 (A–D) or 3 (E) representative experiments of 5 replicates.

treated samples than in vehicle-treated samples. *Pon3* was expressed weakly in HSL-treated samples but was completely negative in vehicle-treated samples. *Pon2* was stably expressed in both groups (Fig. 2E).

DISCUSSION

This study identified HSL as a novel reagent able to promote hair growth in a mouse model of MS. The findings suggest that attenuation of oxidative stress was a mechanism by which HSL treatment enhanced hair growth.

HSL is an interbacterial signaling molecule in *Pseudomonas* quorum sensing, which is a regulatory system that induces the expression of virulence genes depending on bacterial density.[12] PONs protect against bacterial infection by degrading HSL.[13] *Pon2* is dominantly expressed in a wide range of tissues, and *Pon1* and *Pon3* are mainly expressed in the liver and secreted into the serum.[14] We found that *Pon1* was expressed in the skin of MS mice, probably owing to the response to the elevated oxidative stress in MS skin.[6–8] HSL administration slightly increased the expression of *Pon1* and *Pon3* in the skin of *ob/ob* mice. However, elevation in expression of *Pon1* and *Pon3* was not enough to explain the drastic reduction in oxidative stress in hair follicles of *ob/ob* mice. Further studies to examine PON enzyme activities and the expression of other antioxidative enzymes are required.

Because HSL is a small lipid-soluble molecule (approximately 300 Da), it can be delivered topically

and noninvasively to the skin.[15] HSL is a potential novel therapeutic agent for alopecia in MS.

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