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Original Article

Protective effect of human adipose-derived stem cells transplanted to fat grafts against high-power laser therapy mediated fat tissue damage

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ABSTRACT

Introduction: High Power Laser therapy (HPLT) can damage tissues due to its high skin absorption and side effects. The objective of this study was to determine the protective effect of human adipose-derived stem cells (hADSCs) against HPLT-mediated fat tissue damage after fat grafts in an *in vivo* study.
Methods: To determine the viability of fat grafts with transplanted hADSCs, high power laser (HPL) irradiation was performed using a 830 nm gallium–aluminum–arsenide (Ga–Al–As) laser. In the *in vivo* study, fat grafts with hADSCs/Dulbecco's modified Eagle's medium (DMEM) were transplanted and HPL irradiation of each nude mouse was performed per protocol for a period of 13 weeks. Vascular endothelial growth factor (VEGF) and cluster of differentiation 31 (CD31) levels of hADSCs were quantified by immunofluorescent staining using anti-VEGF antibody and anti-CD31 antibody, respectively, along with human mitochondrial (hMT) antibody.
Results: Compared to the HPLT + DMEM group, HPLT + hADSCs group showed increase in the number of viable cells of hADSCs in fat grafts, fat graft survival rate (weights and volumes), and expression levels of VEGF and CD31 in treated nude mice. Results of the *in vivo* study using nude mice and immune cytokine array suggest that hADSCs have potential protective effect against HPLT-mediated fat tissue damage.
Discussion: hADSCs could be applied in clinical fields by inhibiting HPLT-mediated side effects.

1. Introduction

Laser therapy (LT) has been widely used in many clinical situations to accelerate regenerative processes of tissues due to its stimulatory effect on proliferation of various cells.[1] Because LT uses low energy densities and wavelengths, it can easily penetrate into tissues, resulting in photo-bio-modulation effects on cells and tissues.[2] LT has photo-bio-modulation capacity because its photons are absorbed by cytochrome C oxidase in the mitochondrial respiratory chain as an initial trigger of photo-bio-modulation.[3] Increased activity of cytochrome C oxidase in turn increases the production of adenosine triphosphate which can activate injured cells in wounds with low blood perfusion and diverse signaling pathways.[4,5] Low-level laser (light) therapy (LLLT) is a fast-growing technology used to treat a multitude of conditions that require stimulation of healing, relief of pain and inflammation, and restoration of function.[5,6] On the other hand, high-power laser (HPL) therapy used in dermatologic and plastic surgery can induce photo-thermal effect by converting laser light into heat energy. Under normal setting conditions, high power laser therapy (HPLT) has high thermal effect.[7] This is the reason why research studies have basically tested LLLT with energy pulses within milliseconds or continuously emitted energy pulses without producing significant increase in temperature. It has been reported that HPLT has a therapeutic energy window in the range of 2–10 J/cm^2 within wavelength of 810–830 nm to exert effect.[8] The introduction of the HPL in medicine, particularly in the field of dermatology, has resulted in unconceivable progress. However, treatment protocol or evaluation of HPLT for medicine treatment in patients has been established yet.[9] The effect of HPLT-mediated fat tissue damage on the immune system remains largely unknown. For the first time, the potential protective benefit of hADSCs against HPLT-mediated fat tissue damage was demonstrated in *in vivo* using nude mice model.

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2. Materials and methods

2.1. Harvesting ADSCs

Adipose tissues were obtained from the human adipose tissue resected from the transverse rectus abdominis musculocutaneous (TRAM) flaps. Washed and minced adipose tissues were digested with 0.5% type-I collagenase (Worthington, Lakewood, NJ, USA) for 1 h at 37 °C.

2.2. Animals

Six-week-old male nude mice were used. All mice were housed in climate-controlled conditions with a 12-hour light/12-hour dark cycle. They had free access to food and water. All animal experiments conducted in accordance with National Institutes of Health Guide for the Care Use of Laboratory Animals (NIH publication No. 86-23, revised in 1996).

2.3. Experimental designs

Mice were divided into four groups (Table 1). In group 1 (Control), fat grafts with DMEM were transplanted and nude mice were not irradiated with HPLT. In group 2, fat grafts with DMEM were transplanted and nude mice were irradiated with HPLT. In group 3, fat grafts with hADSCs were transplanted and rats were not irradiated with HPLT. In group 4, fat grafts with hADSCs were transplanted and rats were irradiated with HPLT. In groups 2 and 4, all mice were irradiated with 830 nm Ga-Al-As laser used clinically for 30 min once daily for one week for a total of seven times (Fig. 1).

2.4. Weights and volumes of fat grafts

At 13 weeks post transplantation, mice were sacrificed and fat graft weights and volumes were determined. Each fat graft was weighed and the volume of the fat graft was measured using liquid overflow method.[10] After weight measurement, fat graft was subjected to immunofluorescent staining.

2.5. Immunofluorescent staining

At 13 weeks post-transplantation, fat grafts with hADSCs were partially harvested from 10 mice in each group and cell viability was analyzed by immunofluorescent staining using human mitochondrial antibody. Human ADSCs were counted using fluorescence microscopy (Axiovision, Zeiss, Germany). The processed images were analyzed for fluorescence intensity using the ImageJ software (NIH).

2.6. Cytokine array and gene expression in fat graft tissues

Fat graft samples were collected at termination (13 weeks after transplantation) and quickly frozen in liquid nitrogen. A human cytokine array (ARY005; R&D Systems, Minneapolis, MN, USA) was used to screen fat homogenates according to the manufacturer's instructions.

2.7. Statistical analysis

Results are expressed as mean ± standard deviation. Differences for parameters among three treatment groups (groups 2, 3, and 4) were evaluated by analysis of variance (ANOVA) followed by multiple-group comparisons. Unpaired two-tailed *t*-test and Mann-Whitney test were used. *P* value of less than 0.05 was considered statistically significant. SPSS 14.0 for windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

3. Results

3.1. Weights and volumes of fat grafts

Immediately after fat grafting, fat graft weights in groups 1, 2, 3, and 4 were 1.145 ± 0.023, 1.145 ± 0.013, 1.145 ± 0.022, and 1.145 ± 0.012 g, respectively. No significant difference in fat graft weight was found among the four groups. At 13 weeks after fat grafting, fat graft weights in the four groups were 0.811 ± 0.026, 0.681 ± 0.053, 0.981 ± 0.041, 0.852 ± 0.038 g, respectively. At 8 and 13 weeks after grafting, fat graft weights and volumes were significantly decreased in Group 2 (HPLT + DMEM) than those in Group 1 (Control) ($^{*}p < 0.05$; Fig. 2, Table 2). At 4 weeks after grafting, fat graft weights and volumes were slightly decreased in Group 2 than those in Group 1. However, fat graft weights and volumes were significantly increased in Group 4 (HPLT + hADSCs) than those in Group 2 (HPLT + DMEM) at 8 and 13 weeks after grafting ($^{\#}p < 0.05$; Fig. 2, Table 2).

3.2. Cell viability and VEGF levels from grafted hADSCs

At 13 weeks after fat grafting, the number of hADSCs in Group 3 (hADSCs + non-HPLT) was significantly higher than that in Group 1 (Control; $p < 0.05$). Among HPLT groups, Group 4 showed no significant decrease in the number of hADSCs compared to Group 3 at 13 weeks post grafting. However, the number of VEGF markers in Group 3 or 4 was higher than that in Group 1 or 2 (Fig. 3). Among HPLT groups, Group 4 showed no significant decrease in the number of VEGF markers compared to Group 3 at 13 weeks after grafting.

3.3. Cell viability and CD31 levels from grafted hADSCs

At 13 weeks post fat grafting, there was a significant increase in the number of hADSCs in Group 3 (hADSCs + non-HPLT) compared to that in Group 1 (control, $p < 0.05$). Among HPLT groups, Group 4 showed no significant decrease in the number of hADSCs compared to Group 3 (hADSCs + non-HPLT) at 13 weeks post grafting. However, Group 3 or 4 had higher number of CD31 markers compared to Group 1 (Control) or Group 2 (HPLT + DMEM) at the 13-week time point. In addition, Groups 3 and 4 showed had significantly higher number of hADSCs compared to Groups 1 and 2 ($p < 0.05$; Fig. 4). Among HPLT groups, Group 4 showed no significant decrease in the number of CD31 markers compared to Group 3 at the 13-week time point.

Table 1
Laser irradiation protocol for the *in vivo* study.

Group	Fat graft (aspirated fat)	hADSCs treatment	HPLT
1. Control	0.9ml	X (0.1 ml DMEM)	X
2. HPLT + DMEM	0.9ml	X (0.1 ml DMEM)	O
3. hADSCs	0.9ml	O (5×10^6 hADSCs/0.1 ml DMEM)	X
4. HPLT + hADSCs	0.9ml	O (5×10^6 hADSCs/0.1 ml DMEM)	O

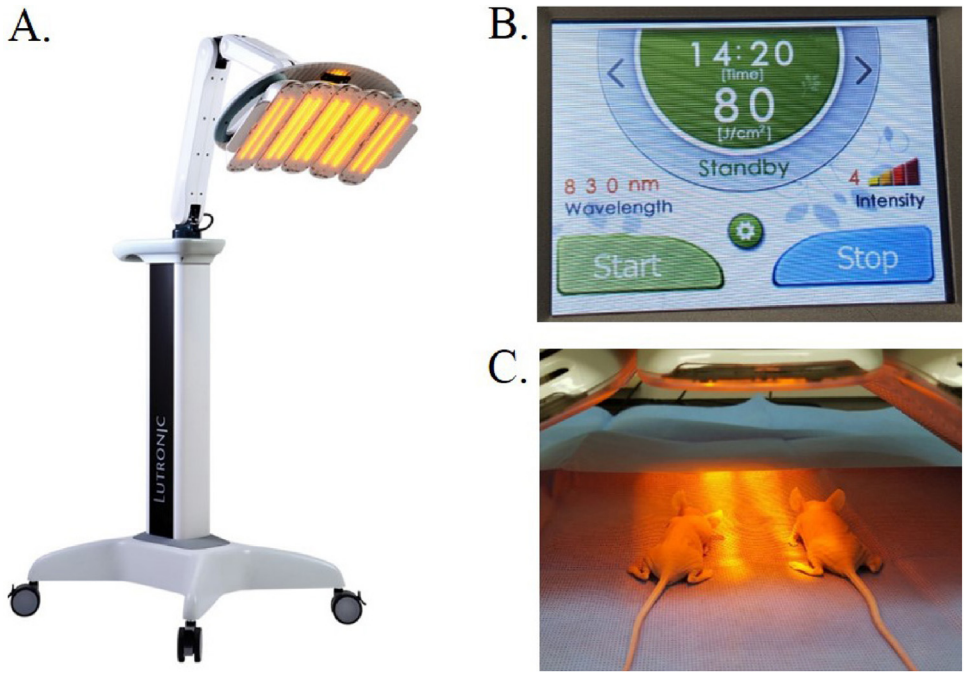

Fig. 1. A and B. 830 nm Ga–Al–As (gallium aluminum arsenide) laser designed for this study. C. In *in vivo* study, low-level laser irradiation for nude mice was performed using 830 nm Ga–Al–As laser.

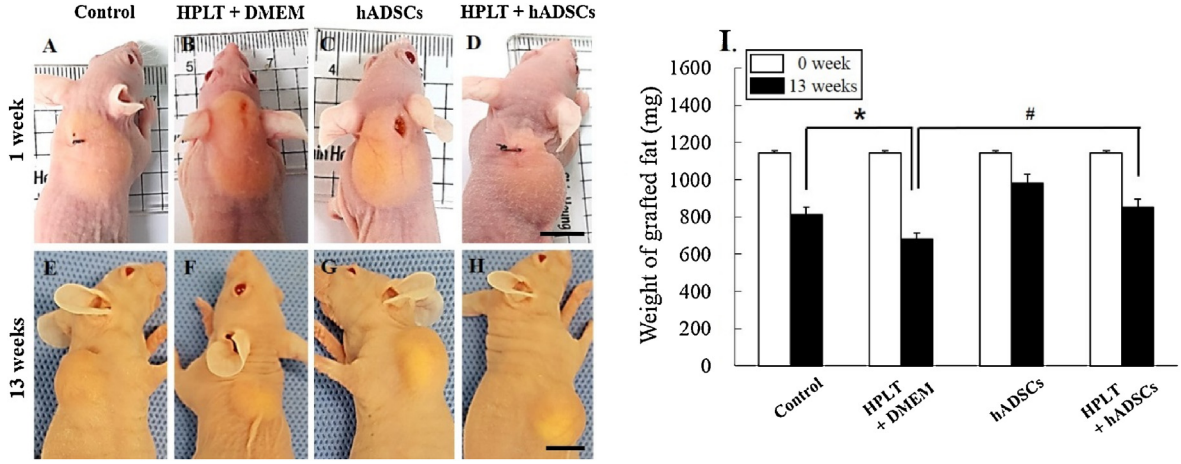

Fig. 2. Comparison of fat graft weights.

3.4. Cytokine profile in fat graft tissues

Cytokine profile in fat homogenate was analyzed to identify potential changes after hADSCs treatment with HPLT (Fig. 5). At 13 weeks post fat grafting, levels of MIF, Serpin E1/Pal-1, and IL-1α were decreased in Group 2 (HPLT + DMEM) compared to those in Group 1 (Control, $^{*}p < 0.05$; Fig. 5). Levels of Serpin E1/Pal-1 and IL-1α were significantly decreased in Group 4 than those in Group 2. However, levels of MIF were significantly increased in Group 4 than those in Group 2 ($^{*}p < 0.05$; Fig. 5).

4. Discussion

Previously, many studies on photo-bio-modulation for stem cell differentiation have mainly used low-level laser therapy and red light to promote differentiation of hADSCs.[11,12] However, little is known about the effect of HPLT for transplanted hADSCs on viability of fat graft. Therefore, the aim of this study was to investigate the protective effect of human ADSCs against HPLT on the viability of fat graft. In this study, the number of hADSCs present in the implanted fat tissue was increased after HPLT. Additionally, VEGF-positive hADSCs were detected in grafted fat (Fig. 3). VEGF is the most specific and effective growth factor that regulates angiogenesis.[13] In this study, relatively low concentrations of MIF, Serpin E1/Pal-1, and IL-1α were detected in fat tissues after fat graft with hADSCs + HPLT or with DMEM + HPLT compared to those after fat graft/hADSCs without irradiation HPLT or fat graft/DMEM group in human cytokine array panel (Fig. 5). Cytokines play important roles in a number of biological processes, including innate immunity, apoptosis, angiogenesis, cell growth, and differentiation.[14] Macrophage MIF (migration inhibitory factor) has been described in the context of delayed-type hypersensitivity.[15] MIF release can be induced by pro-inflammatory factors such as tumor necrosis factor (TNF)-α,

Table 2
Changes of fat graft weights and volumes.

week	Group	Weight (g)	Volume (ml)
0	Control	1.145 ± 0.023	1.03 ± 0.01
	HPLT + DMEM	1.145 ± 0.013	1.01 ± 0.01
	hADSCs	1.145 ± 0.022	1.02 ± 0.02
	HPLT + hADSCs	1.145 ± 0.012	1.01 ± 0.01
4	Control	1.035 ± 0.021	0.98 ± 0.01
	HPLT + DMEM	0.983 ± 0.046	0.93 ± 0.02
	hADSCs	1.001 ± 0.032	0.95 ± 0.02
	HPLT + hADSCs	0.998 ± 0.024	0.94 ± 0.01
8	Control	0.921 ± 0.019	0.87 ± 0.02
	HPLT + DMEM	0.764 ± 0.051*	0.71 ± 0.03*
	hADSCs	0.945 ± 0.021	0.89 ± 0.02
	HPLT + hADSCs	0.897 ± 0.032#	0.84 ± 0.02#
13	Control	0.811 ± 0.026	0.76 ± 0.03
	HPLT + DMEM	0.681 ± 0.053*	0.63 ± 0.05*
	hADSCs	0.981 ± 0.041	0.93 ± 0.02
	HPLT + hADSCs	0.852 ± 0.038#	0.80 ± 0.03#

Values are presented as means ± standard deviation. Control, DMEM + non-HPLT group; HPLT, DMEM + HPLT group; hADSCs, hADSCs + non-HPLT group; HPLT + hADSCs, hADSCs + HPLT group. *$P < 0.05$ compared with the Control group, #$P < 0.05$ compared with the HPLT + DMEM group.

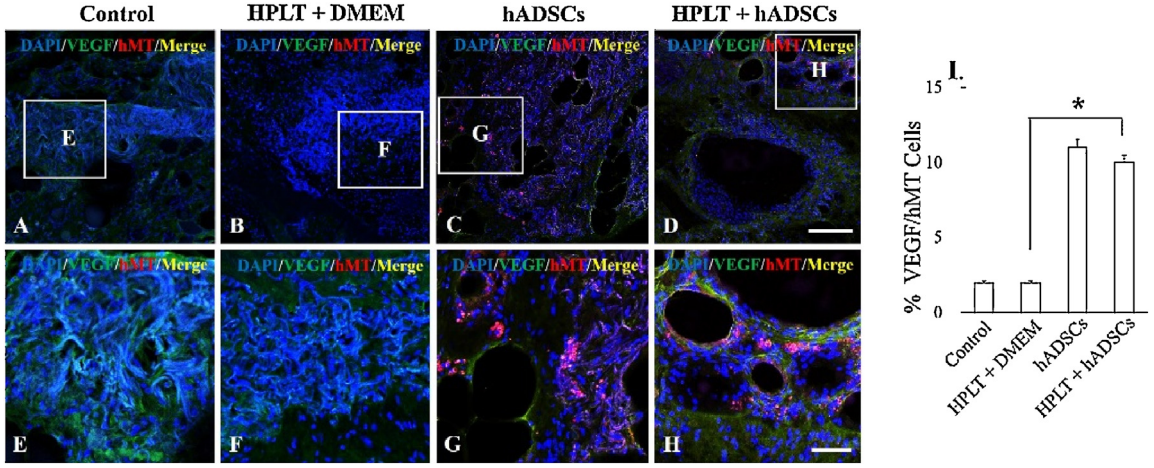

Fig. 3. Expression levels of VEGF and cell viability were quantified by the number of hADSCs. Scale bar = 100 μm (A–D); 25 μm (E–H).

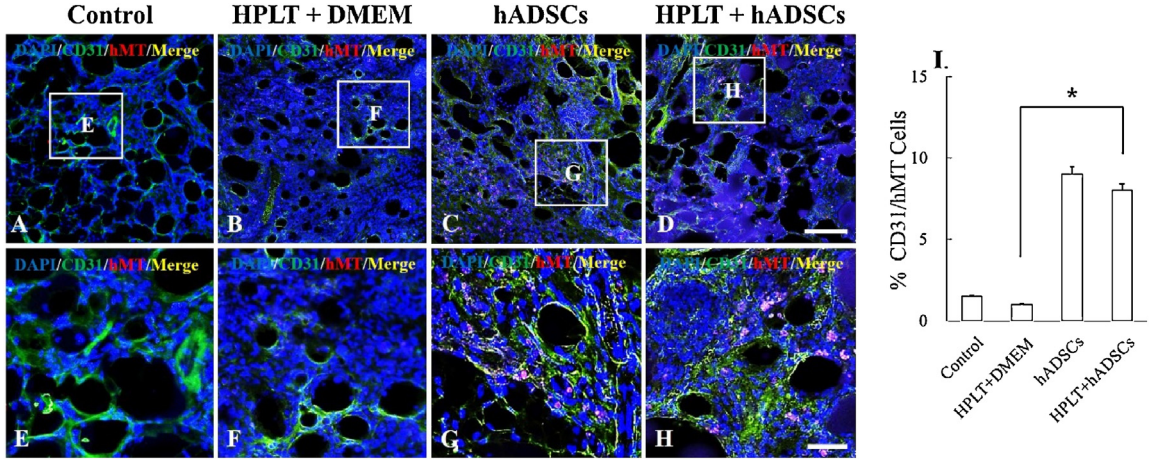

Fig. 4. Expression levels of CD31 and cell viability were quantified by the number of hADSCs. Scale bar = 100 μm (A–D); 25 μm (E–H).

lipopolysaccharide (LPS), and interferon (IFN)-γ.[16] MIF expression in adipose tissue has been reported in epididymal fat pad of rats and murine preadipocyte cell line.[17] MIF mRNA up-regulation is mediated by TNF-α through tyrosine-kinase dependent pathways in cells.[18] Each serpin consists of about 350–400 amino acid residues with molecular masses of 38 to 70 kDa.[19] PAI-1 belongs to inhibitory serpins group (inhibitor of plasminogen activators).[20] Circulating proenzyme plasminogen is cleaved *via* these serine

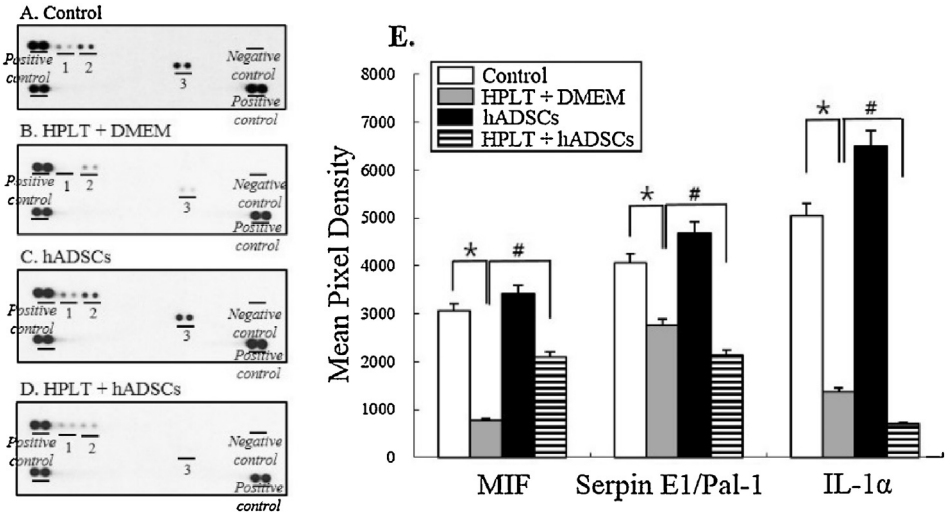

Fig. 5. Inflammatory cytokine expressions in the four groups.

proteases to form active protease plasmin. PAI-1, the most important direct physiological inhibitor of t-PA and u-PA, is a major regulator of the fibrinolytic system.[21] Interleukin-1α (IL-1α) is known to be able to stimulate proliferation of endothelial cells and fibroblasts, thus increasing blood supply at the site of injury.[22] It has also been reported that IL-1α production by cultured peripheral blood mononuclear cells from obese group is significantly elevated compared to that in the control group.[23] In nude mouse model of the present study, IL-1α levels were higher in Group 1 (DMEM + non-HPLT) or Group 3 (hADSCs + non-HPLT) than those in Group 2 (HPLT + DMEM) or Group 4 (HPLT + hADSCs). Results on expression levels of MIF, Serpin E1/Pal-1, and IL-1α in fat homogenates confirmed that fat cell could regulate these three cytokines (Fig. 5). Previously, it has been reported a small increase in stimulation of angiogenesis by a cluster of angiogenic factors in nude mice after fat graft.[24] In addition, there is an immediate decrease in fat cell apoptosis as a potential mechanism.[24] However, the effect of HPLT on the viability of fat graft with hADSCs or the potential mechanism has not been published yet. How cytokines mentioned above either ameliorate or exacerbate the effect of HPLT remains unknown. An interesting finding of this study was that all three fat graft factors (fat grafted weights, volumes, and anti-angiogenic factor) related to injection of hADSCs were higher in the HPLT + human ADSCs group than those in the HPLT group. It has been reported that HPLT may provide future option for treatment. However, individual based variations may indicate that treatments may need to be tailored to individuals. Results of the present study suggest that hADSCs might have potential protective benefit against HPLT-mediated fat tissue damage.

5. Conclusion

HPLT inhibited cell viability of fat grafts. Since hADSCs inhibited HPLT-mediated side effects, it could be applied in clinical fields such as fat grafting or cosmetic procedures and reconstructive surgery. For clinical application, a device that can isolate hADSCs and simultaneously perform HPL irradiation should be developed. This study can be helpful for planning other studies on laser therapy or cell therapy

Conflicts of interest

None.

Acknowledgements

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