Interleukin-1 Beta Regulates Lysophosphatidic Acid-accelerated Skin Wound Healing

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Abstract

Background: Interleukin 1 beta (IL-1β) is considered to be a mediator of infectious, inflammatory and autoimmune diseases, and the kinetics of its production is relevant to understanding the pathogenesis of these diseases. Lysophosphatidic acid (LPA), the structurally simplest bioactive phospholipid, is necessary for homeostasis in various physiological and pathophysiological processes and plays a pivotal role in wound healing. Skin trauma can not only weaken the barrier function, but also cause pain and infection. Chronic wounds are characterized by impaired healing and uncontrolled inflammation that damages the protection of the immune system. The aim of this study is to investigate whether inflammatory factor IL-1β has an effect on LPA in the wound healing model.

Results: In this study, the kinetics of IL-1β gene expression was studied in vivo and in vitro with a wound healing model by quantitative real-time polymerase chain reaction (qRT-PCR) through LPA treatment. As a result, we found that LPA up-regulated inflammatory factor IL-1β in HaCaT cell and skin wound healing. The pro-inflammatory cytokines IL-1β mRNA had higher expression in LPA-treated mice group 3 days after the treatment. In vitro, after the treatment with LPA (20 μM) for 6, 12, and 24 hours, IL-1β mRNA expression increased by 61.16%, 129.39%, and 117.07%, respectively.

Conclusion: These results strongly suggest that IL-1β may regulate LPA-accelerated skin wound healing. IL-1β has significant efficacy, and our observations are of interest to the development of drugs targeting LPA in skin therapy.

Background

Interleukin 1 beta (IL-1β) is an inducible polypeptide with many roles in host defense and homoeostasis. It is also considered to be a mediator of infectious, inflammatory and autoimmune diseases, and the kinetics of its production is relevant to understanding the pathogenesis of these diseases [1]. The healing of human wounds is part of the world's major health problems. Especially, chronic wounds are characterized by impaired healing and uncontrolled inflammation that damages the protection of the immune system and can lead to bacterial infections [2, 3]. Chronic hard-to-heal wounds are usually accompanied by repeated infections, which then result in the necrosis of local tissue, and there is lack of effective treatments, especially for diabetic wounds. This condition wastes medical resources and causes amputation in severe cases [4]. Therefore, wound healing requires efficient and rapid methods to update the current treatments that are often inefficient or limited, thereby meeting the clinical needs.

Lysophosphatidic acid (LPA) is positively correlated to wound healing [5]. LPA is the structurally simplest bioactive phospholipid [6–8], 1-acyl-2-lyso-sn-glycero-3-phosphate [9]. It is endogenous, small molecule, easily soluble in water, not easily degradable, and stable in vivo. LPA activates intracellular signaling pathways through lysophosphatidic acid receptors (LPA_1 ~ LPA_6) [10], is primarily produced by autotaxin (ATX) and catalyzed lysophosphatidylcholine [11, 12], and can be secreted by fat cells, cancer cells, fibroblasts, and platelets. LPA is a bioactive lipid medium necessary for homeostasis in various

physiological and pathophysiological processes. Lysophosphatidic acid receptors mainly exist in the heart and brain and have different biological effects, such as promoting cardiomyocyte hypertrophy [13, 14], having thrombogenic and atherogenic actions [15], being involved in Alzheimer's disease and other neurodegenerative diseases [16, 17], and promoting proliferation, migration and adhesion of ovarian cancer, colon cancer, etc. Previous studies on LPA have mainly focused on cardiovascular and cerebrovascular diseases and cancers, and few studies have been conducted on the skin, involving invasion and metastasis of melanoma [18], fibrosis [19], hair growth [20], and wound healing [21].

Wound healing provokes a series of complex events, including hemostasis, inflammation, proliferation, and remodeling [22, 23]. The inflammatory phase involves activation of the innate immune system. Neutrophils and monocytes are the main cells that migrate rapidly into the wound site after injury, and neutrophils and macrophages release cytokines and growth factors [24]. Neutrophils phagocytose cellular debris and microorganisms, release pro-inflammatory cytokines, chemokines and angiogenic growth factors, and produce reactive oxygen species [25]. Macrophages are the main response cells of LPA, which regulate inflammatory responses [26, 27]. The proliferation phase includes angiogenesis, granulation tissue formation, and re-epithelialization [28]. Collagen fibril deposition and remodeling are important repair processes in the later phase of granulation tissue formation [29]. LPA has been proved to have the functions of promoting the proliferation and migration of fibroblasts and epithelial cells [30]. In addition, LPA can promote the synthesis of laminin 5 in keratinocytes. Laminin 5 may stimulate the adhesion and migration of keratinocytes on the wound beds and form the basement membrane at the dermal epidermal junction to repair the wound [31]. The efficiency of skin wound healing largely depends on the balance between cytokine-mediated pro-inflammatory and pro-regenerative signals [32]. The physiological and pathophysiological effects of LPA in the skin have been gradually explored. Moreover, LPA stimulates the migration of human umbilical cord blood-derived mesenchymal stem cells to promote the wound healing [33], plays a critical role in differentiation and maturation of hair follicle [34], and has a favorable physiological role in oral wound healing [35].

LPA has a function in wound healing, however, the mechanism is still not clear, especially its relationship with inflammatory factors. In this study, we aim to find out the mechanism. We studied the effects of inflammatory factor IL-1β on LPA in wound healing model in vivo, hoping that it may be a new way of wound healing based on LPA. Besides, we investigated the biological effect of LPA and provided a theoretical reference for further study of the mechanism.

Methods

Animals and ethics

All animal protocols were approved by Animal Care Committee of Weifang Medical University (Weifang, China). Female specific pathogen-free (SPF)-grade Kunming mice (KM mice) weighing 18-22 g and aged 4 weeks were purchased from Pengyue Animal Breeding Co., Ltd. (Jinan, China). The 130 mice were random separated to control and experimental groups, and housed in individual cages at a constant

temperature (22°C) and a relative humidity (50% ± 10%) for a 12 h light-dark cycle and were kept in the environment for at least 7 days before the experiments. Throughout the experiment, the animals were provided with food and water.

Mice excisional wound healing model

In this study, a full-thickness excision model of mouse back skin was used. The mouse were first anesthetized under aseptic conditions. The hair of dorsal skin was roughly cleaned using a hair clipper, completely depilated by applying N2S (10 g·L-1), and scrubbed with warm water at 37°C. A skin biopsy punch (ID = 7 mm) was perpendicularly put on the dorsal skin and pushed downward with a circular twisting motion. Next, the middle circular skin was removed with ophthalmic scissors. In this way, a full-thickness excisional circular wound was created. Lysophosphatidic acid (LPA, C21H41O7P·xNa, L7260) was purchased from Sigma (St. Louis, MO, USA). The mice were randomly divided into two groups, i.e., (i) untreated group and (ii) LPA treated group. Each group contained 56 mice. Each animal was housed in a separate cage to prevent further injuries to the wound due to fighting. A Nikon camera (D60) was used to take photos at the specified time at a scale of 1 mm. The degree of wound closure was calculated according to the formula: Wound closure = $(A_0-A_t) / A_0$, where A_0 was the original wound area and A_t was the wound area on day t after the wound.

Histopathological assessments

The mice after experimental modeling were humanely euthanized by CO_2 inhalation in accordance with Code of Practice for the Humane Killing of Animals. The dorsal skin of the mice (n = 8) was promptly fixed with 4% paraformaldehyde for 24 hours, then samples were placed in PBS buffer at 4°C and embedded in paraffin blocks. The wound tissue was sequentially sectioned at 5 μm with a MICROM 17 M325 microtome (Thermo Fisher Scientific, DE). Then the skin sections were stained with H&E to evaluate the presence of necrosis inflammatory cells, hemorrhage, granulation tissue extent, re-epithelialization, and thick epidermis formation. In order to explore the extent of collagen deposition and collagenous fibers in healed tissue during the progress of wound healing, Masson's trichrome staining was performed using Masson's trichrome staining kit (Solarbio, Beijing, China) according to the manufacturer's protocol. An Olympus BX53 microscope (Olympus, UK) was used to take images with the scale bar of 100 μm.

RNA isolation and quantitative real time polymerase chain reaction

Total RNA was extracted from wound skin tissue of mice (n = 8) using 20 μM LPA or control on days 0, 3, 6, 9, 12 and 15, and total RNA was extracted from human HaCaT cells using 0, 5, 10 and 20 μM LPA, and cultured in a serum-free medium for 6 hours, 12 hours and 24 hours using TRIzol reagent (CWBIO, Beijing, China). 2 μg of total mRNA was reverse transcripted to cDNA in a 20 μL volumn using ReverTra Ace qPCR RT Kit (TOYOBO, Qsaka, Japan) at 37°C for 15 mins and 98°C for 5 mins. Then cDNA was used in the quantitative real-time polymerase chain reaction (qRT-PCR) to analyze the relative expression of mRNA using SYBR® Green Realtime PCR Master Mix (TOYOBO, Qsaka, Japan) according to the manufacturer's

protocol. The mRNA expression levels were normalized to 18s rRNA for mice samples and GAPDH for HaCaT cells. The following primers (Qingdao Personal Gene Biotechnology Co., Ltd, Qingdao, China) were used in the reaction: IL-1β (human) forward: 5'-ACAGATGAAGTGCTCCTTCCA-3', reverse: 5'-GTCGGAGATTCGTAGCTGGAT-3'; IL-6 (human) forward: 5'-GGTGTTGCCTGCTGCCTTCC-3', reverse: 5'-GTTCTGAAGAGGTGAGTGGCTGTC-3'; GAPDH (human) forward: 5'-AGAAGGCTGGGGCTCATTTG-3', reverse: 5'-AGGGGCCATCCACAGTCTTC-3'; IL-1β (mouse) forward: 5'-TCGCAGCAGCACATCAACAAGAG-3', reverse: 5'-TGCTCATGTCCTCATCCTGGAAGG-3'; 18s rRNA (mouse) forward: 5'-TTGACTCAACACGGGAAACC-3', reverse: 5'-AGACAAATCGCCCACCAACACC-3'. We preincubated the qRT-PCR conditions at 95°C for 30 seconds, then used LightCycler® 480 II (Roche Diagnostics International Ltd, Switzerland) to apply 40 cycles of 95°C for 5 seconds, 55°C for 10 seconds, and 72°C for 15 seconds. The relative IL-1β and IL-6 mRNA expressions were calculated using the $2^{-\Delta\Delta Cq}$ method [36].

Cell culture and proliferation detection

A colorimetric assay was used to measure the proliferation of immortalized human HaCaT keratinocyte cells (Kunming Institute of Zoology, CAS, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, AU) and penicillin (100 U/ml)-streptomycin (100 mg/ml) (Solarbio, Beijing, China) at 37°C in a humidified atmosphere of 5% CO_2. HaCaT cells (2×10^4 cells/well, 180 μL) were plated into 96-well plates (Corning Incorporated, NY, USA). After adhering to the plate, the cells were incubated with vehicle (DMEM) or LPA at different concentrations of 3.125, 6.25, 12.5, 25, 50 and 100 μM for 24 hours. Then 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution (Solarbio, Beijing, China) was added to each well for a further 4 hour incubation at 37°C. After the cells were washed 3 times with PBS (pH 7.4), the insoluble formazan product was dissolved by incubating with 150 μL DMSO. The absorbance of each well was measured on an enzyme-linked immunosorbent assay (ELISA) microplate reader at 490 nm. The optical density reflects the level of cellular metabolic activity. Each experiment was performed in quintuplicate.

Cell migration assay

The Ibidi culture inserts (Ibidi, Martinsried, DE) were placed in a 24-well culture plate (Corning Incorporated, NY, USA). Each side were seeded with 15,000 HaCaT cells and then cultured with 5% CO_2 at 37°C for 24 hours. The inserts were removed and the cells were washed three times with PBS to remove cell debris. Cells were cultured at 37°C in the medium of 20 μM LPA and the untreated cells were used as control. A microscope (4× lens, Olympus IX71, UK) was used to take photographs at the beginning (0 hour) and every 6 hours for 48 hours. Jmicro Vision was used to measure the width of the cell-free area at each time point at the scale bar of 500 μm.

Statistical analysis

The collected data was analyzed using GraphPad Prism 8.0.2 software. All data were expressed as mean ± SD. One-way ANOVA with post hoc analysis was performed to analyze the data, and $p < 0.05$ was considered statistically significant.

Results

LPA accelerated wound healing

General morphology of the wounds was obtained for all treatment groups, as shown in Figure 1. The wound area of each group decreased over time. Compared with the group treated with saline, the wound closure of mice treated with LPA (20 μM) was significantly faster, and there was no phenomenon of inflammation or infection [37].

LPA improved the quality of newly formed skin tissue

In general, LPA (20μM) treatment can lead to re-epithelialization with a well-defined epidermis, as shown in the cross-sections from the wound tissues at day 15. The higher magnification of the sections further confirmed the good organization of these structures (Fig. 2A). Moreover, compared with control group, wounds treated with LPA showed a higher content of collagen, which were well organized in fibrils. This can be proved by the higher amount of the blue color in the Masson's Trichrome staining (Fig. 2A and B) [38, 39]. Overall, these results indicated that LPA was able to accelerate the wound healing and improve the quality of the newly formed skin tissue [40].

The dynamic alteration of IL-1β in vivo

Many factors can affect the speed of wound healing, especially cytokines [41]. A robust host inflammatory response is effective in isolating and controlling infection, as well as repairing the affected tissues [42]. On the contrary, prolonged inflammation is harmful and may lead to the deregulated stages of the wound healing [24]. Wound healing can be improved by dampening inflammation [43]. Both acute and chronic inflammation can hinder wound healing, leading to either chronic ulceration or scarring, presenting multiple derangements [44]. To investigate the effect of LPA on wound healing speed in the animal model, IL-1β was detected in this study. The pro-inflammatory cytokines IL-1β mRNA had higher expression in the mice treated by LPA for 3 days. However, compared with the saline treated group, both of these cytokines showed a steady decrease on days 6, 9, 12 and 15 (Fig. 3) [1, 45]. IL-1β was up-regulated in wound healing model of LPA treatment.

LPA promoted HaCaT cell proliferation

MTT assay was performed to investigate whether LPA can promote HaCaT cell proliferation. The effects of LPA on the proliferation of HaCaT cells are shown in Figure 4. The result showed that LPA had a dominant simulative effect on HaCaT cell proliferation [40]. The promotion rate of the proliferation of HaCaT cells was dependent on the concentration of LPA. At concentrations of 3.125, 6.25, 12.5 and 25

μM, the rates of proliferation of the HaCaT cells were increased by 17.41%, 19.94%, 28.35% and 10.56%, respectively.

Effects of LPA on HaCaT cell migration

The effects of LPA on the migratory capacity of HaCaT cells were determined by their ability to induce in vitro wound closure. Wound healing was simulated by the fusion of monolayer cells and the cells were incubated for 48 hours. Compared with the control group and the Vitamin C group, the group treated with LPA (20 μM) showed better migration of HaCaT cells (Fig. 5A and B) [46]. In other words, LPA can significantly influence cell migration.

Kinetics of IL-1β gene expression in vitro

To further explore the working principle of LPA, we stimulated HaCaT cells with or without LPA, and determined IL-1β (Fig. 6A-C) and IL-6 (Fig. 6D-F) at different time points after stimulation [45]. Compared with the control group, the IL-1β mRNA expression increased by 61.16%, 129.39% and 117.07% after 6, 12 and 24 hours of LPA (20 μM) treatment, respectively. Interestingly, compared with the control group, IL-6 mRNA expression increased by 33.04% and 291.69% after 6 and 12 hours of LPA (20 μM) treatment, respectively. However, after 24 hour LPA treatment, IL-6 mRNA expression decreased by 71.97%.

Discussion

This study aimed to investigate the effects of the inflammatory factor IL-1β on LPA in wound healing model. We investigated the kinetics of IL-1β gene expression in vivo and in vitro. Those findings suggested that IL-1β can regulate LPA- accelerated skin wound healing. The early inflammatory response helps to promote the wound healing by releasing some cytokines at the wound site, such as IL-1β, IL-6, etc. [1, 47, 48]. We found the LPA treatment group had higher IL-1β gene expression in early inflammatory response.

The inflammatory response plays an important role in the entire wound healing process. The skin is the largest organ of the human body, which can effectively prevent the invasion of harmful substances and keep the body in a relatively stable state [38]. Any unrepaired breach of the skin may impair its barrier function and expose human tissues to microbial infections and mechanical damage [49]. Underlying disease process, hypoxia of the wound bed, wound infection, and increased level of inflammatory mediators predispose wounds to become chronic [50]. Once skin damage occurs, the body will produce a violent oxidative stress reaction and inflammatory response to counteract the adverse factors in the external environment to accelerate wound healing [46, 51, 52]. Some drugs [53, 54] can also promote wound healing by regulating inflammatory factors. Pro-inflammatory cytokines may be relevant to acute pain after surgical incision in the rat plantar [55].

Our works showed that LPA in vivo had many advantages in skin penetration, shortening repair time, and increasing subcutaneous collagen, and had a significant efficacy on wound healing. Meanwhile, we also

found that LPA up-regulated inflammatory factor IL-1β in HaCaT cell during skin wound healing. The proliferation and migration of epidermal cells participate in epidermal regeneration and promote wound healing. HaCaT cells are human immortalized epidermal cells, non-tumor-derived human normal skin immortalized keratinocytes, and their differentiation characteristics are similar to human normal keratinocytes. We used MTT assay and cell migration assay to detect the proliferation effects of LPA on HaCaT cells, and found that after the treatment with LPA (12.5 and 25 μM), the proliferation rate of HaCaT cells was increased by 28.35%, 10.56%, respectively. In addition, compared with the control group, the repair rate of scratch with 20 μM LPA in 48 hour increased 57.56%. These results showed that LPA can significantly promote the proliferation and migration of HaCaT cells and may promote skin wound healing.

The growth factor such as phospholipid lysophosphatidic acid (LPA) and its analogs cyclic phosphatidic acid (cPA) are both natural phospholipid mediator [12, 56]. cPA and LPA induce hyaluronic acid in human skin fibroblasts mainly by activating LPA_1-$G_{i/o}$, phosphatidylinositol 3-kinase (PI3K), extracellular signal regulated kinase (ERK), and cyclic adenosine monophosphate response element-binding protein (CREB) signaling pathway [56]. Hyaluronic acid is a predominant component of the extracellular matrix and is involved in cellular functions such as skin hydration, joint lubrication, and stimulating the migratory response of keratinocytes in wound healing [45].

Wound healing in mice is very similar to that in humans, although human skin and mouse skin have an anatomical difference, because mouse skin contains a subcutaneous panniculus carnosus muscle. We use mice as a model of human wound healing, because mice are often used as a model to investigate cytokines in vivo [57]. H&E staining and Masson's trichrome staining exhibited that the mice treated with LPA had higher re-epithelization and collagen deposition compared to the control. These results showed that LPA significantly accelerate wound healing [58].

More and more in vivo and in vitro evidences show that LPA is highly involved in skin disease [59-63]. Investigating the role of LPA in skin wound healing, skin scar, cutaneous melanoma, scleroderma, itchy skin, allergic dermatitis, skin barrier, cutaneous pain and hair growth is helpful to understand the physiological and pathophysiological role of LPA in skin. LPA is a bioactive phospholipid messenger involved in multiple physiological and pathophysiological processes, and is a necessary bioactive lipid medium to maintain homeostasis in many physiological and pathophysiological processes in the skin through different cell signaling pathways.

In summary, IL-1β regulates LPA-accelerated skin wound healing. LPA is an effector compound, which has potential wound healing ability by not only promoting migration and proliferation of keratinocytes, but also regulating IL-1β. Further researches on the mechanism of LPA will help to explore its role in skin therapy and develop drugs targeting LPA.

Abbreviations

LPA, Lysophosphatidic acid; qRT-PCR, quantitative real time polymerase chain reaction; IL-1β, interleukin-1 beta; ATX, autotaxin; IL-6, interleukin-6; cPA, cyclic phosphatidic acid; PI3K, phosphatidylinositol 3-kinase; ERK, extracellular signal regulated kinase; CREB, cyclic adenosine monophosphate response element-binding protein

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XYY developed hypotheses. XYY, YYG and XY designed experiments. DLG, XXM and LYL analyzed and interpreted data and wrote the draft. DLG, XXM, LYL, YWL, HJ, JJX and QYG performed experiments. XYY, YYG and XY interpreted data and critically reviewed the paper. All the authors approved the final version of the manuscript.

Ethics approval and consent to participate

All experimental procedures involving animals were approved by the Animal Care Committee of Weifang Medical University, Weifang, China. (approval no. Y136/2018).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

[1] Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev. 2018;281(1):8-27.

[2] Saleh B, Dhaliwal HK, Portillo-Lara R, Shirzaei Sani E, Abdi R, Amiji MM, et al. Local Immunomodulation Using an Adhesive Hydrogel Loaded with miRNA-Laden Nanoparticles Promotes Wound Healing. Small. 2019;15(36).

[3] Saleh K, Stromdahl AC, Riesbeck K, Schmidtchen A. Inflammation Biomarkers and Correlation to Wound Status After Full-Thickness Skin Grafting. Front Med (Lausanne). 2019;6:159.

[4] Bumpus K, Maier MA. The ABC's of wound care. Curr Cardiol Rep. 2013;15(4):346.

[5] Lei L, Su J, Chen J, Chen W, Chen X, Peng C. The role of lysophosphatidic acid in the physiology and pathology of the skin. Life Sci. 2019;220:194-200.

[6] Zhang J, Li Y, Wang C, Wang Y, Zhang Y, Huang L, et al. Lysophosphatidic Acid Induces Apoptosis of PC12 Cells Through LPA1 Receptor/LPA2 Receptor/MAPK Signaling Pathway. Front Mol Neurosci. 2020;13:16.

[7] Gu C, Wang F, Zhao Z, Wang H, Cong X, Chen X. Lysophosphatidic Acid Is Associated with Atherosclerotic Plaque Instability by Regulating NF-kappaB Dependent Matrix Metalloproteinase-9 Expression via LPA2 in Macrophages. Front Physiol. 2017;8:266.

[8] Srikanth M, Chew WS, Hind T, Lim SM, Hay NWJ, Lee JHM, et al. Lysophosphatidic acid and its receptor LPA1 mediate carrageenan induced inflammatory pain in mice. European Journal of Pharmacology. 2018;841:49-56.

[9] Chen H, Liu S, Liu X, Yang J, Wang F, Cong X, et al. Lysophosphatidic Acid Pretreatment Attenuates Myocardial Ischemia/Reperfusion Injury in the Immature Hearts of Rats. Frontiers in Physiology. 2017;8:153.

[10] Miyabe C, Miyabe Y, Nagai J, Miura NN, Ohno N, Chun J, et al. Abrogation of lysophosphatidic acid receptor 1 ameliorates murine vasculitis. Arthritis Res Ther. 2019;21(1):191.

[11] Tokumura A, Majima E, Kariya Y, Tominaga K, Kogure K, Yasuda K, et al. Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional

phosphodiesterase. J Biol Chem. 2002;277(42):39436-42.

[12] Benesch MG, Tang X, Venkatraman G, Bekele RT, Brindley DN. Recent advances in targeting the autotaxin-lysophosphatidate-lipid phosphate phosphatase axis in vivo. J Biomed Res. 2016;30(4):272-84.

[13] Cai L, Fan G, Wang F, Liu S, Li T, Cong X, et al. Protective Role for LPA3 in Cardiac Hypertrophy Induced by Myocardial Infarction but Not by Isoproterenol. Front Physiol. 2017;8:356.

[14] Yang J, Xu J, Han X, Wang H, Zhang Y, Dong J, et al. Lysophosphatidic Acid Is Associated With Cardiac Dysfunction and Hypertrophy by Suppressing Autophagy via the LPA3/AKT/mTOR Pathway. Front Physiol. 2018;9:1315.

[15] Schober A, Siess W. Lysophosphatidic acid in atherosclerotic diseases. Br J Pharmacol. 2012;167(3):465-82.

[16] Ramesh S, Govindarajulu M, Suppiramaniam V, Moore T, Dhanasekaran M. Autotaxin-lysophosphatidic acid signaling in alzheimer's disease. Int J Mol Sci. 2018;19(7):1827.

[17] Shi J, Dong Y, Cui MZ, Xu X. Lysophosphatidic acid induces increased BACE1 expression and Abeta formation. Biochim Biophys Acta. 2013;1832(1):29-38.

[18] Muinonen-Martin AJ, Susanto O, Zhang Q, Smethurst E, Faller WJ, Veltman DM, et al. Melanoma cells break down LPA to establish local gradients that drive chemotactic dispersal. PLoS Biol. 2014;12(10):e1001966.

[19] Castelino FV, Bain G, Pace VA, Black KE, George L, Probst CK, et al. An Autotaxin/Lysophosphatidic Acid/Interleukin-6 Amplification Loop Drives Scleroderma Fibrosis. Arthritis Rheumatol. 2016;68(12):2964-74.

[20] Khan GM, Hassan N, Khan N, Humayun M, Khan K, Khaliq S, et al. Biallelic mutations in the LPAR6 gene causing autosomal recessive wooly hair/hypotrichosis phenotype in five Pakistani families. Int J Dermatol. 2019;58(8):946-52.

[21] Demoyer JS, Skalak TC, Durieux ME. Lysophosphatidic acid enhances healing of acute cutaneous wounds in the mouse. Wound Repair Regen. 2000;8(6):530-7.

[22] Watterson KR, Lanning DA, Diegelmann RF, Spiegel S. Regulation of fibroblast functions by lysophospholipid mediators: potential roles in wound healing. Wound Repair Regen. 2007;15(5):607-16.

[23] Gonzalez AC, Costa TF, Andrade ZA, Medrado AR. Wound healing - A literature review. An Bras Dermatol. 2016;91(5):614-20.

[24] Shukla SK, Sharma AK, Gupta V, Yashavarddhan MH. Pharmacological control of inflammation in wound healing. J Tissue Viability. 2019;28(4):218-22.

[25] Weimann E, Silva MBB, Murata GM, Bortolon JR, Dermargos A, Curi R, et al. Topical anti-inflammatory activity of palmitoleic acid improves wound healing. PLoS One. 2018;13(10):e0205338.

[26] Chang CL, Lin ME, Hsu HY, Yao CL, Hwang SM, Pan CY, et al. Lysophosphatidic acid-induced interleukin-1 beta expression is mediated through Gi/Rho and the generation of reactive oxygen species in macrophages. J Biomed Sci. 2008;15(3):357-63.

[27] Ray R, Rai V. Lysophosphatidic acid converts monocytes into macrophages in both mice and humans. Blood. 2017;129(9):1177-83.

[28] Shedoeva A, Leavesley D, Upton Z, Fan C. Wound Healing and the Use of Medicinal Plants. Evid Based Complement Alternat Med. 2019;2019:2684108.

[29] Wang T, Feng Y, Sun H, Zhang L, Hao L, Shi C, et al. miR-21 regulates skin wound healing by targeting multiple aspects of the healing process. Am J Pathol. 2012;181(6):1911-20.

[30] Leeuwen FNv, Giepmans BNG, Meeteren LAv, Moolenaar WH. Lysophosphatidic acid: mitogen and motility factor. Biochem Soc T. 2003;31:1209-12.

[31] Amano S, Akutsu N, Ogura Y, Nishiyama T. Increase of laminin 5 synthesis in human keratinocytes by acute wound fluid, inflammatory cytokines and growth factors, and lysophospholipids. Br J Dermatol. 2004;151(5):961-70.

[32] Nosenko MA, Ambaryan SG, Drutskaya MS. Proinflammatory Cytokines and Skin Wound Healing in Mice. Molecular Biology. 2019;53(5):653-64.

[33] Ryu JM, Han HJ. Autotaxin-LPA axis regulates hMSC migration by adherent junction disruption and cytoskeletal rearrangement via LPAR1/3-dependent PKC/GSK3beta/beta-catenin and PKC/Rho GTPase pathways. Stem Cells. 2015;33(3):819-32.

[34] Inoue A, Arima N, Ishiguro J, Prestwich GD, Arai H, Aoki J. LPA-producing enzyme PA-PLA(1)alpha regulates hair follicle development by modulating EGFR signalling. EMBO J. 2011;30(20):4248-60.

[35] Thorlakson HH, Engen SA, Schreurs O, Schenck K, Blix IJS. Lysophosphatidic acid induces expression of genes in human oral keratinocytes involved in wound healing. Arch Oral Biol. 2017;80:153-9.

[36] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25:402-8.

[37] Sun T, Zhan B, Zhang W, Qin D, Xia G, Zhang H, et al. Carboxymethyl chitosan nanoparticles loaded with bioactive peptide OH-CATH30 benefit nonscar wound healing. Int J Nanomedicine. 2018;13:5771-86.

[38] Silva JP, Dhall S, Garcia M, Chan A, Costa C, Gama M, et al. Improved burn wound healing by the antimicrobial peptide LLKKK18 released from conjugates with dextrin embedded in a carbopol gel. Acta

Biomater. 2015;26:249-62.

[39] Li X, Fan R, Tong A, Yang M, Deng J, Zhou L, et al. In situ gel-forming AP-57 peptide delivery system for cutaneous wound healing. Int J Pharm. 2015;495(1):560-71.

[40] Qin D, Lee WH, Gao Z, Zhang W, Peng M, Sun T, et al. Protective effects of antioxidin-RL from Odorrana livida against ultraviolet B-irradiated skin photoaging. Peptides. 2018;101:124-34.

[41] Kim HS, Sun X, Lee JH, Kim HW, Fu X, Leong KW. Advanced drug delivery systems and artificial skin grafts for skin wound healing. Adv Drug Deliv Rev. 2019;146:209-39.

[42] Gonzalez CD, Ledo C, Cela E, Stella I, Xu C, Ojeda DS, et al. The good side of inflammation: Staphylococcus aureus proteins SpA and Sbi contribute to proper abscess formation and wound healing during skin and soft tissue infections. Biochim Biophys Acta Mol Basis Dis. 2019;1865(10):2657-70.

[43] Rosique RG, Rosique MJ, Farina Junior JA. Curbing Inflammation in Skin Wound Healing: A Review. Int J Inflam. 2015;2015:316235.

[44] Bradshaw A, Sylakowski K, Wells A. The Pro-reparative Engine: Stem Cells Aid Healing by Dampening Inflammation. Curr Pathobiol Rep. 2018;6(2):109-15.

[45] Kondo T, Ohshima T. The dynamics of inflammatory cytokines in the healing process of mouse skin wound: a preliminary study for possible wound age determination. Int J Legal Med. 1996;108:231-6.

[46] Chereddy KK, Her CH, Comune M, Moia C, Lopes A, Porporato PE, et al. PLGA nanoparticles loaded with host defense peptide LL37 promote wound healing. J Control Release. 2014;194:138-47.

[47] Zepter K, Häffner A, Soohoo LF, De Luca D, Tang HP, Fisher P, et al. Induction of biologically active IL-1 beta-converting enzyme and mature IL-1 beta in human keratinocytes by inflammatory and immunologic stimuli. J Immunol. 1997;159(12):6203-8.

[48] Gao ZH, Deng CJ, Xie YY, Guo XL, Wang QQ, Liu LZ, et al. Pore-forming toxin-like protein complex expressed by frog promotes tissue repair. FASEB J. 2019;33(1):782-95.

[49] Walter MN, Wright KT, Fuller HR, MacNeil S, Johnson WE. Mesenchymal stem cell-conditioned medium accelerates skin wound healing: an in vitro study of fibroblast and keratinocyte scratch assays. Exp Cell Res. 2010;316(7):1271-81.

[50] Atkin L. Chronic wounds: the challenges of appropriate management. Br J Community Nurs. 2019;24:S26-S32.

[51] Sun BK, Siprashvili Z, Khavari PA. Advances in skin grafting and treatment of cutaneous wounds.Science. 2014;346:941-5.

[52] Nolte M, Margadant C. Controlling Immunity and Inflammation through Integrin-Dependent Regulation of TGF-beta. Trends Cell Biol. 2020;30(1):49-59.

[53] Moghadam SE, Moridi Farimani M, Soroury S, Ebrahimi SN, Jabbarzadeh E. Hypermongone C Accelerates Wound Healing through the Modulation of Inflammatory Factors and Promotion of Fibroblast Migration. Molecules. 2019;24(10).

[54] Mi B, Liu J, Liu G, Zhou W, Liu Y, Hu L, et al. Icariin promotes wound healing by enhancing the migration and proliferation of keratinocytes via the AKT and ERK signaling pathway. Int J Mol Med. 2018;42(2):831-8.

[55] Chen X, Zhang B, Li J, Feng M, Zhang Y, Yao W, et al. Celastrol attenuates incision-induced inflammation and pain associated with inhibition of the NF-kappaB signalling pathway via SARM. Life Sci. 2018;205:136-44.

[56] Maeda-Sano K, Gotoh M, Morohoshi T, Someya T, Murofushi H, Murakami-Murofushi K. Cyclic phosphatidic acid and lysophosphatidic acid induce hyaluronic acid synthesis via CREB transcription factor regulation in human skin fibroblasts. Biochim Biophys Acta. 2014;1841(9):1256-63.

[57] Saghazadeh S, Rinoldi C, Schot M, Kashaf SS, Sharifi F, Jalilian E, et al. Drug delivery systems and materials for wound healing applications. Adv Drug Deliv Rev. 2018;127:138-66.

[58] Mu L, Tang J, Liu H, Shen C, Rong M, Zhang Z, et al. A potential wound-healing-promoting peptide from salamander skin. FASEB J. 2014;28(9):3919-29.

[59] Sato M, Shegogue D, Hatamochi A, Yamazaki S, Trojanowska M. Lysophosphatidic acid inhibits TGF-beta-mediated stimulation of type I collagen mRNA stability via an ERK-dependent pathway in dermal fibroblasts. Matrix Biol. 2004;23(6):353-61.

[60] Krawczyk M, Milkiewicz M, Marschall HU, Bartz C, Grunhage F, Wunsch E, et al. Variant adiponutrin confers genetic protection against cholestatic itch. Sci Rep. 2014;4:6374.

[61] Allanore Y, Distler O, Jagerschmidt A, Illiano S, Ledein L, Boitier E, et al. Lysophosphatidic Acid Receptor 1 Antagonist SAR100842 for Patients With Diffuse Cutaneous Systemic Sclerosis: A Double-Blind, Randomized, Eight-Week Placebo-Controlled Study Followed by a Sixteen-Week Open-Label Extension Study. Arthritis Rheumatol. 2018;70(10):1634-43.

[62] Susanto O, Koh YWH, Morrice N, Tumanov S, Thomason PA, Nielson M, et al. LPP3 mediates self-generation of chemotactic LPA gradients by melanoma cells. J Cell Sci. 2017;130(20):3455-66.

[63] Zeng C, Wen B, Hou G, Lei L, Mei Z, Jia X, et al. Lipidomics profiling reveals the role of glycerophospholipid metabolism in psoriasis. Gigascience. 2017;6(10):1-11.

Figures

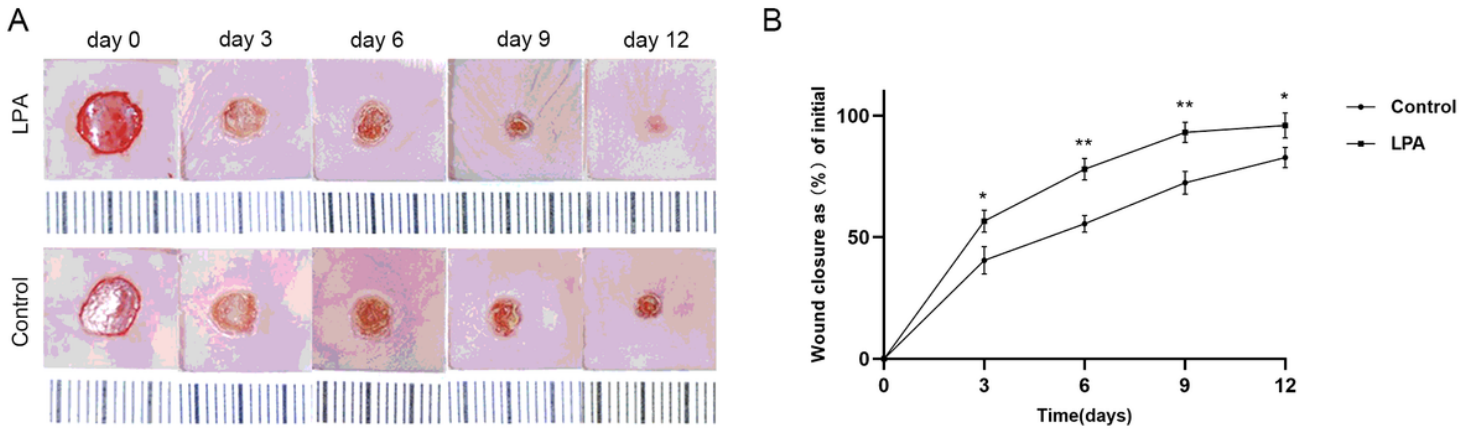

Figure 1

Topical application of LPA accelerated the healing of full-thickness wounds in mice. (A) Images of a representative mouse from each group 0, 3, 6, 9 and 12 days after the wound was created. Scale bar = 1 mm. (B) Quantitative analysis of wound closure by Image J software based on the images in A. Data are expressed as mean ± SD (n = 8/group), *P < 0.05, **P < 0.01 vs. control.

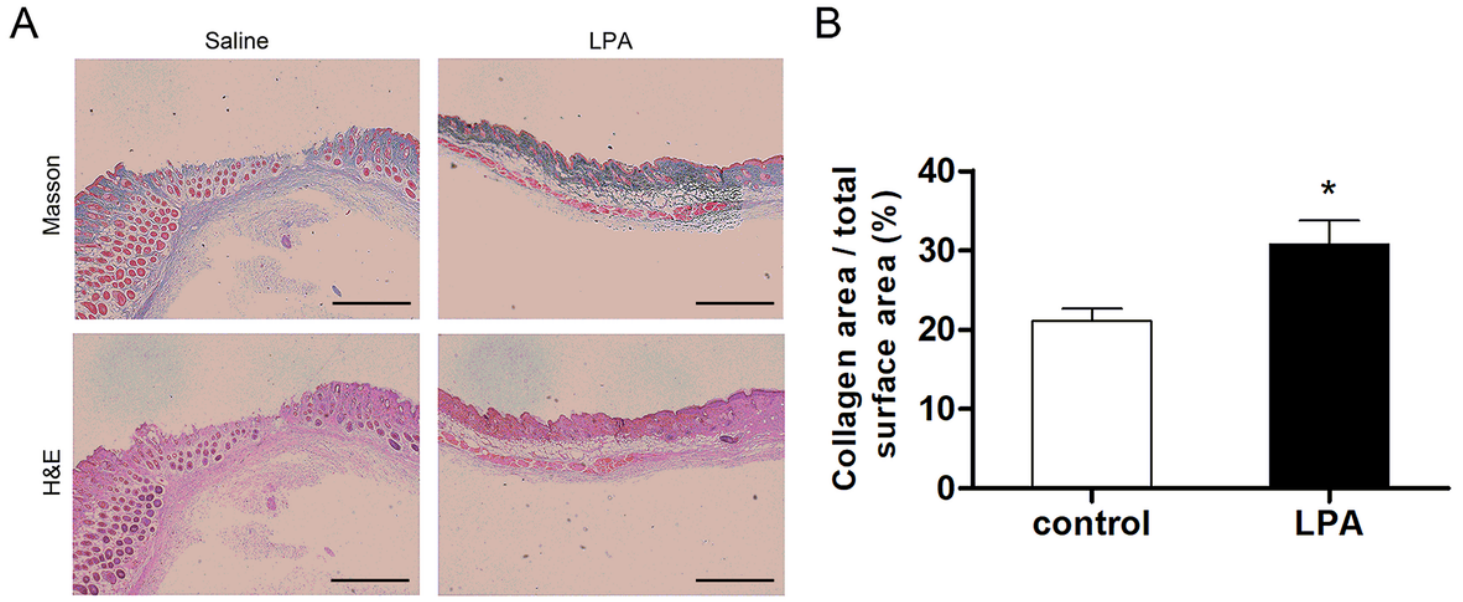

Figure 2

Histological evaluation of wound cross-sections. (A) Tissues on the 15th day after wound was creased were stained using Masson's Trichrome and Hematoxylin and Eosin. Higher collagen content (blue) with well-formed fibrils was observed in the LPA-treated tissues. Scale bar = 100 μm. (B) Determination of the surface area of the blue positive collagen stained by Masson's trichrome and collagen ratio from wounds in mice using Image J software. Data are expressed as mean ± SD (n = 8/group), *P < 0.05, **P < 0.01 vs. control.

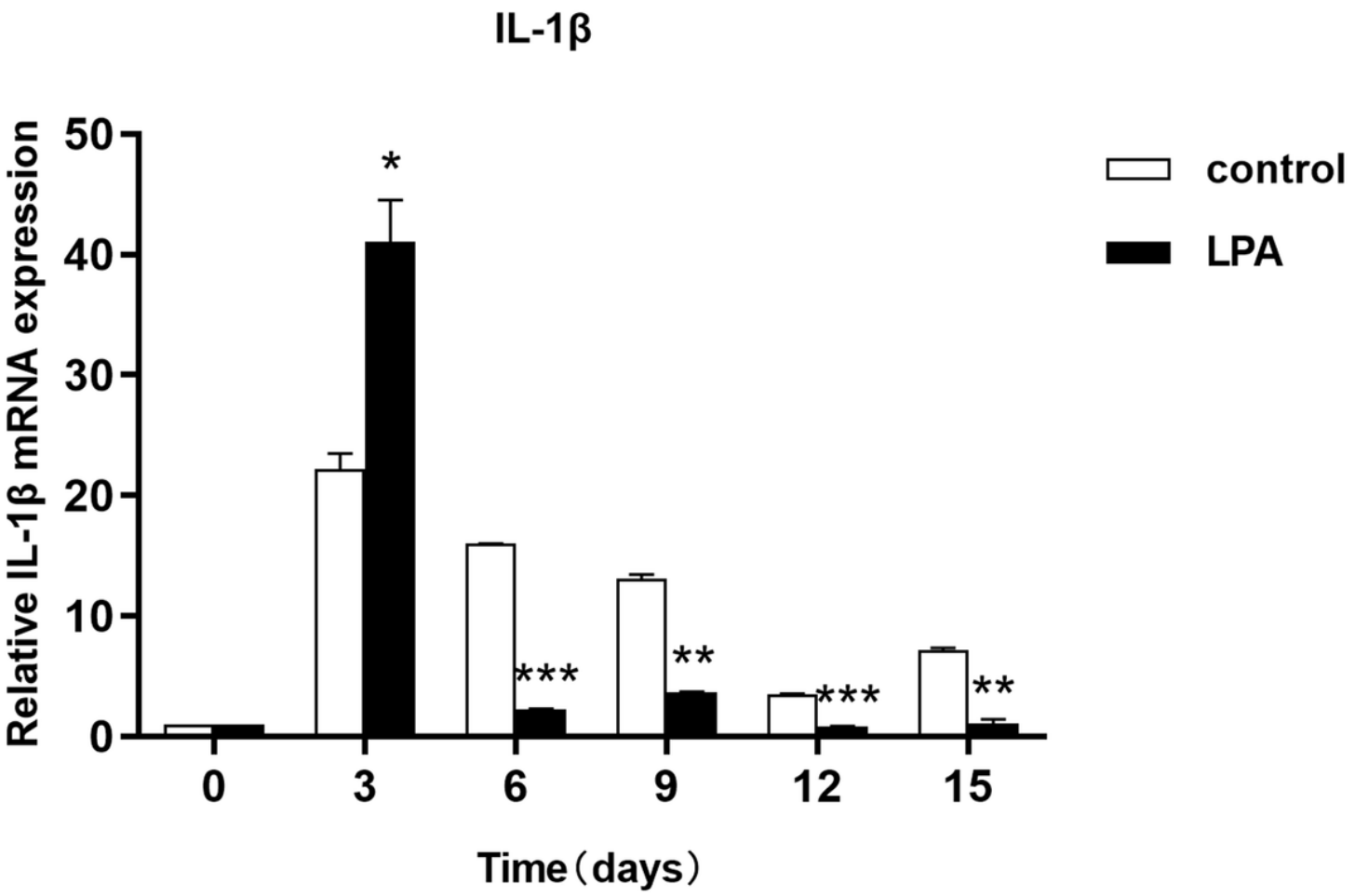

Figure 3

Effects of LPA on the expression of IL-1β mRNA in mice. Total RNA was extracted from wound skin tissue of mice (n = 8) with 20 μM LPA treatment. The expression of IL-1β mRNA was measured three times. Data are expressed as mean ± SD (n = 8/group), *P < 0.05, **P < 0.01 vs. control.

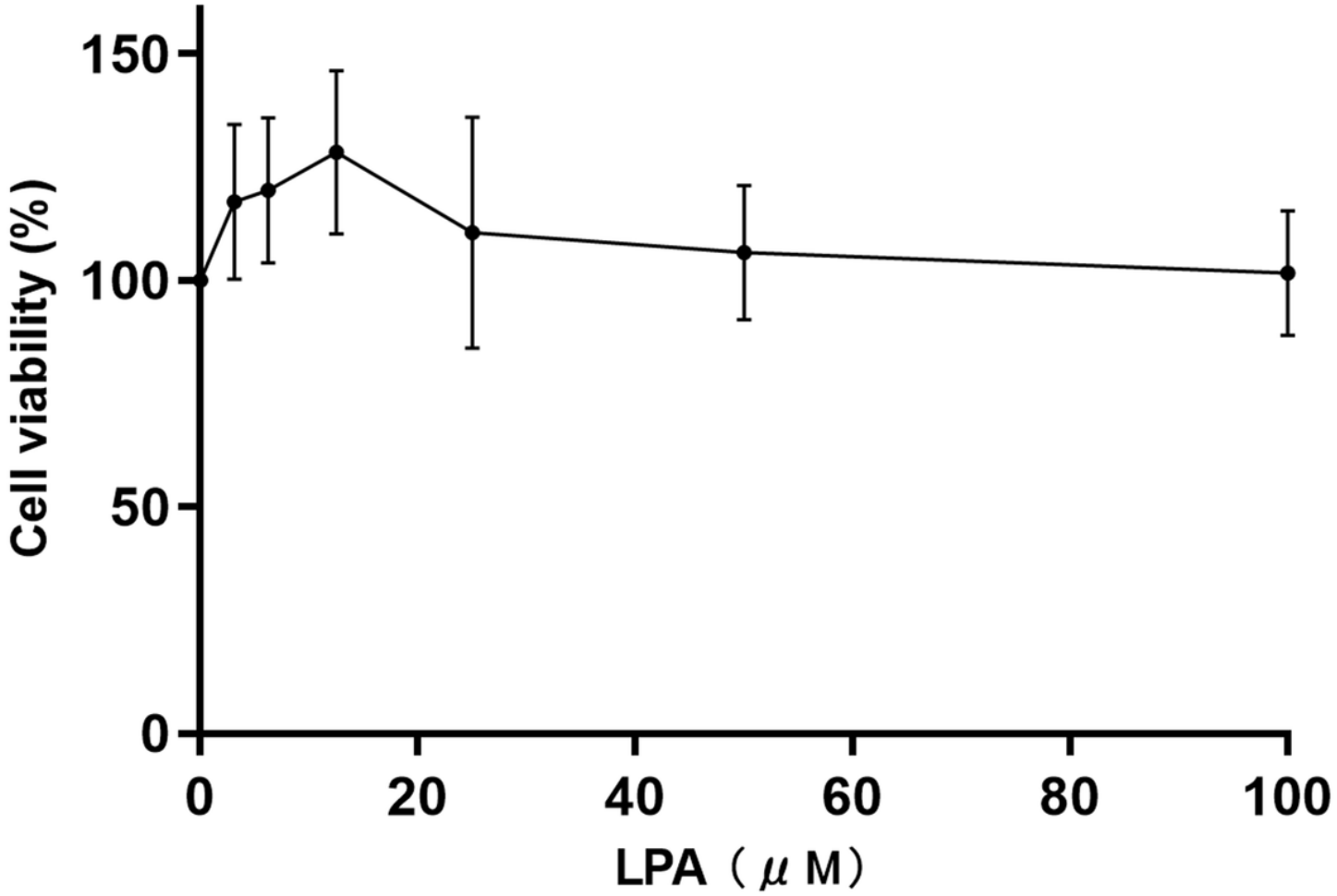

Figure 4

Concentration dependence of the promotion effect of LPA on the proliferation of HaCaT cells. The proliferation of HaCaT cells was measured using MTT assay at the LPA concentrations of 0, 3.125, 6.25, 12.5, 25, 50, and 100 μM. The cell viability was measured three times. Data are expressed as mean ± SD (n = 3), *P < 0.05, **P < 0.01 vs. control.

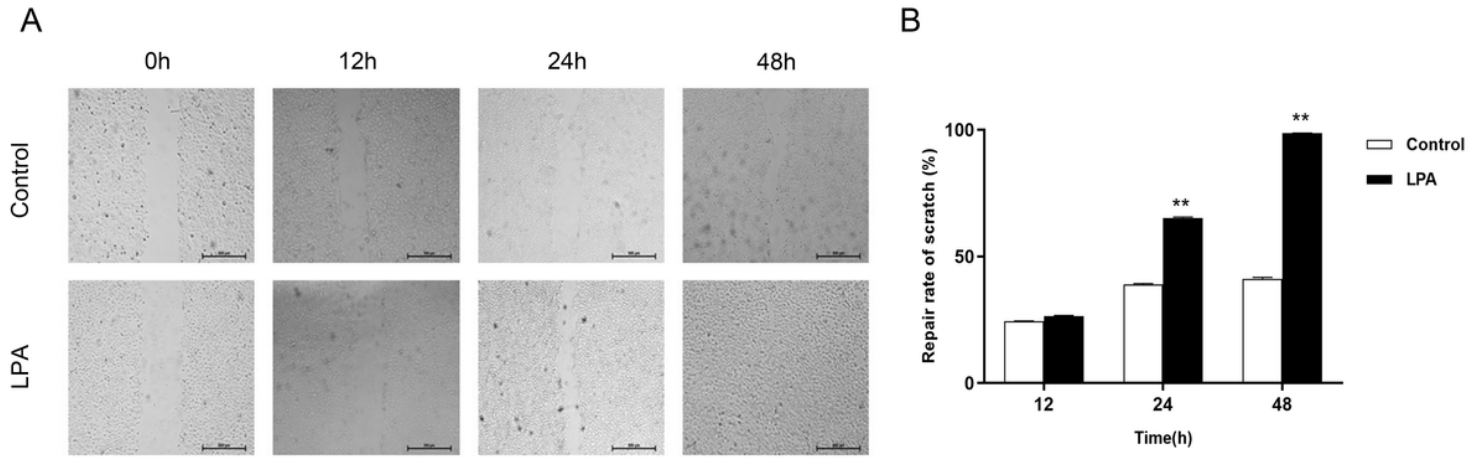

Figure 5

Effects of LPA on cell migration and cumulative wound closure rate. (A) Effects of LPA on the migration of HaCaT cells were analyzed by IBIDI cell migration assays. Scale bar = 500 μm. (B) The results were expressed as the percentage of the initial wound area calculated by Image J software based on A. Data are expressed as mean ± SD (n = 3), *P < 0.05, **P < 0.01 vs. control.

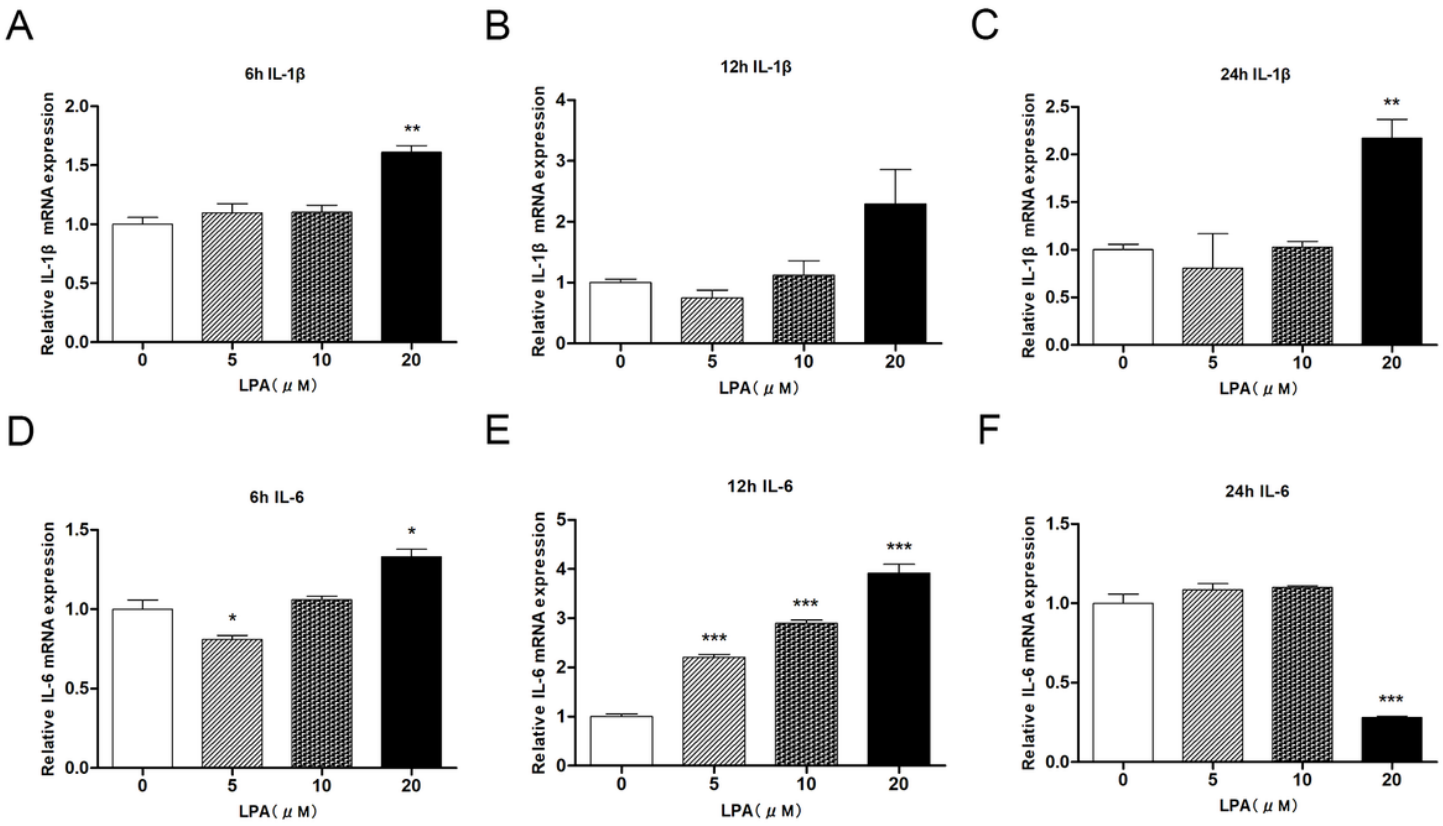

Figure 6

Effects of LPA on the expression of mRNA in vitro. Total RNA was extracted from human HaCaT cells treated with LPA. (A) IL-1β mRNA expression was measured 6 hours after injury. (B) IL-1β mRNA expression was measured 12 hours after injury. (C) IL-1β mRNA expression was measured 24 hours after injury. (D) IL-6 mRNA expression was measured 6 hours after injury. (E) IL-6 mRNA expression was measured 12 hours after injury. (F) IL-6 mRNA expression was measured 24 hours after injury. Each sample for each gene was measured three times. Data are expressed as mean ± SD (n = 3), *P < 0.05, **P < 0.01 vs. control.

Supplementary Files

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