

## Research Article

# Loratadine, an H<sub>1</sub> Antihistamine, Inhibits Melanogenesis in Human Melanocytes

Hye-Rim Moon <sup>1</sup>, Soo Youn Jo,<sup>2</sup> Hak Tae Kim,<sup>3</sup> Woo Jin Lee,<sup>3</sup> Chong Hyun Won,<sup>3</sup> Mi Woo Lee,<sup>3</sup> Jee Ho Choi,<sup>3</sup> and Sung Eun Chang <sup>3</sup>

<sup>1</sup>Department of Dermatology, Korea University Ansan Hospital, Korea University, 123, Jeokgeum-ro, Danwon-gu, Ansan-si, Gyeonggi-do 15355, Republic of Korea

<sup>2</sup>Asan Institute for Life Science, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Republic of Korea

<sup>3</sup>Department of Dermatology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Republic of Korea

Correspondence should be addressed to Sung Eun Chang; csesnumd@gmail.com

Received 27 September 2018; Revised 31 December 2018; Accepted 1 February 2019; Published 17 March 2019

Academic Editor: Emiliano Antiga

Copyright © 2019 Hye-Rim Moon et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

It has long been believed that histamine is associated with cutaneous melanogenesis. Specifically, H<sub>2</sub>-receptor antagonists reportedly inhibit melanogenesis, but H<sub>1</sub>-receptor antagonists, which are some of the most commonly prescribed medicines in dermatology, have not been studied to determine whether and how they regulate melanogenesis. Therefore, we screened H<sub>1</sub>-receptor antagonists to determine whether they inhibit melanogenesis and found that loratadine was particularly effective, in this regard without compromising cellular viability. Loratadine downregulated microphthalmia-associated transcription factor (MITF) and tyrosinase in melanocytes. To determine the intracellular signaling pathways, Akt was consistently activated by loratadine. PI3K/Akt pathway inhibitor, LY294002, restored the reduced melanin content that was induced by loratadine. In addition, phospho-GSK-3 $\beta$  also was found to be increased following loratadine treatment. Loratadine reduced the amount of PKC- $\beta$ II in the membrane fraction, thereby decreasing its activity. Taken together, our data indicate that loratadine regulates melanogenesis via Akt/MITF and PKC- $\beta$ II signaling, thereby leading to the inhibition of melanogenic proteins. The antimelanogenic effects of loratadine have potentially significant and useful roles in dermatologic practice, although further clinical studies will be required to test this.

## 1. Introduction

Hyperpigmentary skin disorders are a major issue of concern for people with dark skin. Until now, clinicians have attempted to treat hyperpigmentary disorders using various therapeutic modalities, including hypopigmenting agents, but the results have been discouraging.

Diverse types of immune cells have been thought to play an important role in melanogenesis. Various inflammatory dermatoses can induce postinflammatory hyperpigmentation by affecting the morphologic change and functional activity of melanocyte. We recently reported the inflammatory features of melasma in Korean women and that mast cells are frequently found in the dermis of melasma lesions [1]. In addition, we proposed the association between increased

dermal mast cells and clinical features of hyperpigmentation and telangiectasia in photodamaging process [2]. Therefore, inflammatory mediators such as histamine, prostaglandins, and nitric oxide have been identified as possible therapeutic approaches for hyperpigmentary skin disorders [3].

Histamine is a ubiquitously distributed inflammatory mediator that is released from tissue mast cells and blood basophils, thereby inducing various inflammatory skin disorders. Since the cutaneous actions of histamine as a melanogen were first reported [4], there have been studies on the role of histamine in skin hyperpigmentation [3, 5, 6]. Histamine-induced morphologic changes in human melanocytes and increased tyrosinase activity, resulting in increased melanogenesis and H<sub>2</sub> antihistamines specifically, suppressed

the stimulatory effects of histamine [5, 6]. However, the involvement of histamine in melanogenesis via other receptors such as H<sub>1</sub>, H<sub>3</sub>, and H<sub>4</sub> receptors remains unclear. Especially considering common use of H<sub>1</sub> antihistamines in dermatologic practice, it is remarkable that little has been reported about the effects of H<sub>1</sub> antihistamines on melanogenesis. Therefore, we tried to focus on defining the effects of H<sub>1</sub> antihistamines on melanogenesis.

## 2. Materials and Methods

**2.1. Materials.** Loratadine, 3,4-dihydroxy-L-phenylalanine (L-DOPA), cholera toxin (CT), 12-O-tetradecanoylphorbol-13-acetate (TPA), and antibodies specific to  $\beta$ -actin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Antibody specific for Akt (#2920S), phosphor-Akt (ser473, #4058S),  $\beta$ -catenin (#9562L), GSK-3 $\beta$  (27C10, #9315), phospho-GSK-3 $\beta$  (Ser9, #9336), p44/42 MAPK (Erk1/2) (#9102S), and p44/42 MAPK (Erk1/2) (Thr202/Tyr204, #9101S) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies specific for tyrosinase (C-19) and PKC- $\beta$ II (C-18) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Microphthalmia (MITF) Ab-1 (C5, MS-771-P0) was obtained from Neomarkers (Fremont, CA, USA).

**2.2. Cell Culture.** Normal human melanocytes (NHM) were obtained from Invitrogen and maintained in Medium 254 (Cascade Biologics, Portland, OR, USA), which contained human melanocyte growth supplement at 37°C in a 5% CO<sub>2</sub> incubation [7]. The Mel-Ab cell line is a mouse-derived, spontaneously immortalized melanocyte cell line that synthesizes large quantities of melanin. Mel-Ab cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 nM TPA, 1 nM CT, 1% antibiotic-antimycotic solution (100X) at 37°C in 5% CO<sub>2</sub>. B16F10 cells were maintained in DMEM supplemented with 10% FBS and 1% antibiotic-antimycotic solution (100X) at 37°C in 5% CO<sub>2</sub> [8].

**2.3. Cell Viability Assay.** Cell viability of NHM and Mel-Ab cells was measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Duchefa, Netherlands) assay. NHM and Mel-Ab cells were seeded at the same density in 24-well plate. After incubation with loratadine for 24 hr. MTT was prepared as 2.5 mg/ml stock solution in phosphate buffered saline (PBS) and stored at 4°C. Then, stock MTT solution was added at 200  $\mu$ l/well and the plates incubated at 37°C for 30 min to 4 hr. Thereafter, MTT solution was removed. After addition of 3 ml of DMSO the plates were incubated for 15 min at 37°C to dissolve the formazan crystals. Absorbance was measured at 570 nm using an enzyme immunosorbent assay (ELISA) reader (Molecular Devices Co., Sunnyvale, CA). The reference wavelength was 560-650 nm.

**2.4. Melanin Contents and Microscopy.** NHM and Mel-Ab cells were treated with specific agonists and for 5 days or 3 days. Cells were dissolved in 550  $\mu$ l of 1 N NaOH at

100°C for 30 min and centrifuged at 13,000 rpm for 5 min. The optical density (OD) of the supernatants was measured at 405 nm using a microplate reader. Before measuring the melanin content, the cells were observed under a phase contrast and photographed for microscope (Olympus, Tokyo, Japan).

**2.5. Tyrosinase Activity.** NHM and Mel-Ab cells were seeded in 6-well plates and incubated with loratadine for 5 days or 3 days. The cells were washed with ice-cold PBS and lysed with phosphate buffer (pH 6.8) containing 1% Triton X-100. The cells were then disrupted by freezing and thawing, and the lysates were clarified by centrifugation at 15000 rpm for 10 minutes. After quantifying the protein levels of the lysate and adjusting the protein concentrations with lysis buffer, 90  $\mu$ l of each lysate containing the same amount of protein was placed in each well of a 96-well plate, and 10  $\mu$ l of 10 mM L-DOPA was then added to each well. The control wells contained 90  $\mu$ l of lysis buffer and 10  $\mu$ l of 10 mM L-DOPA. Following incubation at 37°C, absorbance was measured every 10 min for at least 1 hr at 475 nm using a microplate reader.

**2.6. Cell Fractionation.** We used a cell fractionation kit from cell signaling. Cell fractionation was performed according to the manufacturer's instructions.

**2.7. Western Blot Analysis.** Cells were lysed in protein lysis buffer and centrifuged at 13,000 rpm for 30 min. The protein concentration was determined using a Bradford protein assay. 20  $\mu$ g of protein per lane was separated by SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes, which were then saturated with 5% skim milk in Tris-buffered saline containing 0.5% Tween 20. Blots were incubated with the appropriate primary antibodies following the manufacturer's data sheet. Image analysis was used to determine the relative band densities, which was performed using Image J software (<https://imagej.nih.gov/ij/>).

**2.8. Total RNA Extraction and cDNA Synthesis.** Total cellular RNA was extracted from the NHM using the Favor Prep™ Total RNA purification mini according to the manufacturer's instructions (Favorgen, Ping Tung, Taiwan). Following isolation, the quantity and quality of the RNA were determined using a NanoDrop ND-1000 spectrophotometer (ND-1000, NanoDrop Technologies, Inc. Wilmington, DE, USA). Single-stranded complementary DNA (cDNA) was synthesized from 1  $\mu$ g of total RNA using RevertAid First Strand cDNA Synthesis kit according to the manufacturer's instructions (Thermo Scientific, Rockford, IL, USA).

**2.9. Real-Time RT-PCR.** qRT-PCR was performed using the LightCycler® 480II machine coupled with SYBR Green chemistry (Roche Applied Science, Indianapolis, IN, USA). In terms of qRT-PCR settings, initial denaturation was performed at 95°C for 5 min, followed by amplification at 95°C for 10 sec, 60°C for 10 sec, and 72°C for 10 sec for 45 cycles. The cDNA obtained was amplified with the following primers:

RPLP0, Forward 5'-GGCGACCTGGAAGTCCAACT-3', Reverse 5'-CCATCAGCACCACAGCCTTC-3'. MITF, Forward 5'-ACTTTCCTTATCCCATCCACC-3', Reverse 5'-TGAGATCCAGAGTTGTCGTACA-3'. Primers specific for RPLP0 were used for loading control amplifications.

**2.10. Statistics.** The statistical significance of the differences between groups was assessed using analysis of variance (ANOVA), followed by the Student's *t*-test. In this study, *p* < 0.05 is considered significant.

### 3. Results

**3.1. Loratadine, an H<sub>1</sub>- Receptor Antagonist, Suppresses Melanogenesis in NHM and Mel-Ab Cells.** Even though H<sub>2</sub>-receptor agonists and antagonists have been extensively studied previously, the effect of H<sub>1</sub>-receptor antagonists on melanogenesis has not been fully understood. First, we explored whether H<sub>1</sub>-receptor antagonists influenced the melanogenesis in B16F10 cells. Among the H<sub>1</sub>-receptor antagonists screened, ebastine, clemisole, terfenadine, and loratadine significantly decreased the melanin content (Table 1 and Supplementary Fig. 1A). We selected ebastine and loratadine as they decreased the melanin content in a dose-dependent manner. While ebastine affected cellular viability in NHM and Mel-Ab cells (data was not shown), loratadine showed a dose-dependent response without affecting cellular viability in NHM and Mel-Ab cells (Figures 1(a) and 1(c)). Also, loratadine treatment decreased the tyrosinase activity in a dose-dependent manner (Figure 1(b)).

To determine the involvement of H<sub>1</sub>-receptor agonist on melanogenesis, we evaluated the effects of 2-pyridylethylamine, an H<sub>1</sub>-receptor agonist. The H<sub>1</sub> receptor agonist did not affect the melanin content in Mel-Ab cells or reverse the loratadine induced melanin reduction (Supplementary Figure 1B and 1C).

**3.2. Loratadine Inhibits Expression Levels of MITF in NHM.** As loratadine decreased melanin synthesis and tyrosinase activity, we next determined whether loratadine affected the expression of MITF, which plays crucial role in tyrosinase gene expression in melanogenesis. Loratadine significantly decreased the protein level of MITF and tyrosinase at 12 hr (Figure 2(a)). Furthermore, MITF and tyrosinase mRNA level also decreased after loratadine treatment (Figure 2(b)). These results indicated that loratadine reduced melanogenesis through the downregulation of MITF signaling pathway.

**3.3. Inhibitory Effects of Loratadine on Melanogenesis Were Partially Associated with Akt Phosphorylation in NHM.** To investigate the intracellular signaling pathways, expression levels of  $\beta$ -catenin, phospho-GSK-3 $\beta$ , phospho-Akt, and phospho-ERK were detected in NHM (Figure 3(a)). To determine the effect of loratadine on PI3K/Akt/GSK-3 $\beta$  signaling pathway, phosphorylation of Akt and GSK-3 $\beta$  was found to be increased following loratadine treatment,

markedly at 30 min.  $\beta$ -catenin and phospho-ERK were not altered.

We next evaluated whether LY294002, a selective inhibitor of PI3K, affected the inhibitory effect of loratadine on melanogenesis. As shown in Figure 3(b), loratadine monotreatment significantly reduced the melanin content, whereas cotreatment with LY294002 and loratadine reversely increased the melanin content in NHM. Our results revealed that antimelanogenic effect of loratadine in NHM is associated with activation of PI3K/Akt/GSK-3 $\beta$  signaling pathway.

**3.4. Antimelanogenic Effects of Loratadine Were Associated with Membrane PKC- $\beta$ II in NHM.** H<sub>1</sub>-receptor is one of the G protein-coupled receptors that couples to G $\alpha_{q/11}$  proteins, leading to the activation of the calcium/protein kinase C (PKC) pathway. Since, specifically, PKC- $\beta$ II is activator of tyrosinase, we investigated whether loratadine could decrease activity of PKC- $\beta$ II. As shown in Figure 3(c), loratadine treatment reduced the amount of PKC- $\beta$ II in the membrane fraction, thereby decreasing its activity. The molecular mechanisms of antimelanogenic effect of loratadine demonstrated above are summarized in Figure 4.

### 4. Discussion

Histamine acts through 4 different classes of receptors (H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub>, and H<sub>4</sub> receptors) on effector cells. Among these 4 receptors, H<sub>1</sub> and H<sub>2</sub> receptors have been extensively studied and demonstrated well-known proallergic properties [9]. Since the first synthesized in 1937, H<sub>1</sub> antihistamines have been some of the most commonly prescribed medicine in dermatologic conditions, including allergic reactions, urticaria, atopic dermatitis, and pruritus. Antihistamines were considered histamine receptor antagonists, but they actually work as inverse agonists by binding to histamine receptors, thereby returning cellular equilibrium and reducing allergic property [10].

Moreover, H<sub>1</sub> and H<sub>2</sub> receptors exist on the surface of human melanocytes and melanoma cells [11]. Yoshida et al. [6] have reported that histamine induces melanogenetic effects on human melanocytes by accumulating cyclic adenosine monophosphate (cAMP) and subsequently activating protein kinase A (PKA), especially via the H<sub>2</sub> receptor. Kim et al. [12] investigated the signaling pathways involved in histamine-induced melanocyte proliferation and melanogenesis. They showed that an H<sub>2</sub> antihistamine, famotidine, suppressed the effects induced by the H<sub>2</sub> agonist, amthamine, and histamine itself. Histamine stimulated melanocyte proliferation and melanogenesis via the H<sub>2</sub> receptor and Erk, CREB, or Akt activation [12]. More recently, it was reported that the H<sub>2</sub> receptor mediated growth-differentiation factor-15 (GDF-15) could be involved in histamine-induced melanogenesis [13]. In an *in vivo* study, the UVB-induced hyperpigmentation of guinea pig skin was suppressed by topically applying an H<sub>2</sub> antihistamine [14].

Although the H<sub>1</sub> receptor is a major therapeutic target of inflammatory skin disorders, there have been few

TABLE I: Summary of the H1 antihistamines screened for antimelanogenesis. Among them, clemisole, ebastine, loratadine, and terfenadine significantly decreased the melanin content (text with \*).

Name	Trade name	Therapeutic chemical class	Systematic (IUPAC) name	Molecular weight
<b>First generation antihistamines</b>				
Brompheniramine maleate	Bromfed, Dimetapp, Bromfenex, Dimetane, Lodrane	Alkylamine	3-(4-bromophenyl)- <i>N,N</i> -dimethyl-3-pyridin-2-yl-propan-1-amine	435.3213
Chlorpheniramine maleate	Chlor-trimeton	Alkylamine	3-(4-chlorophenyl)- <i>N,N</i> -dimethyl-3-pyridin-2-yl-propan-1-amine	390.8703
Clemastine fumarate	Tavist	Aminoalkyl ethers	(2 <i>R</i> )-2-[2-[(1 <i>R</i> )-1-(4-chlorophenyl)-1-phenylethoxy]ethyl]-1-methylpyrrolidine	459.9746
Clemizole hydrochloride*	-	-	1-[(4-chlorophenyl)methyl]-2-(pyrrolidin-1-ylmethyl)benzimidazole	362.3053
Diphenhydramine hydrochloride	Benadryl, unisom, sominex, zzzquil	Aminoalkyl ethers	2-(diphenylmethoxy)- <i>N,N</i> -dimethylethanamine	291.824
Doxylamine succinate	unisom	Aminoalkyl ethers	( <i>RS</i> )- <i>N,N</i> -dimethyl-2-(1-phenyl-1-pyridin-2-yl-ethoxy)-ethanamine	388.4677
Methapyrilene hydrochloride	-	Substituted ethylenediamines	<i>N,N</i> -dimethyl- <i>N'</i> -pyridin-2-yl- <i>N'</i> -(2-thienylmethyl)ethane-1,2-diamine	297.8526
Orphenadrine hydrochloride	Norflex, Banflex, Orphenate, Flexoject, Flexon	Aminoalkyl ethers	<i>N,N</i> -dimethyl-2-[(2-methylphenyl)-phenyl-methoxy]-ethanamine	305.8511
Pheniramine maleate	Avil	Substituted alkylamines	<i>N,N</i> -dimethyl-3-phenyl-3-pyridin-2-yl-propan-1-amine	356.4253
Promethazine hydrochloride	Phenergan	Phenothiazine derivatives	( <i>RS</i> )- <i>N,N</i> -dimethyl-1-(10 <i>H</i> -phenothiazin-10-yl)propan-2-amine	320.8873
Pyrilamine maleate	Pyrex	Substituted ethylene diamines	<i>N</i> -(4-methoxybenzyl)- <i>N',N'</i> -dimethyl- <i>N</i> -pyridin-2-ylethane-1,2-diamine	401.4664
Triprolidine hydrochloride	Actidil, Mydil, Actifed	other	2-[( <i>E</i> )-1-(4-methylphenyl)-3-pyrrolidin-1-yl-prop-1-enyl]pyridine	314.8616
<b>Second generation antihistamines</b>				
Ebastine*	Evasfin, kestine, ebastel, aleva, ebatrol	Other	4-(4-benzhydryloxy-1-piperidyl)-1-(4-tert-butylphenyl)butan-1-one	469.6731
Cetirizine dihydrochloride	Zirtec, Zyrtec, reactine	Piperazine derivatives	(±)-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid	461.8199
Epinastine hydrochloride	Alesion, Elestat, Purivist, Relestat	other	( <i>RS</i> )-3-amino-9,13b-dihydro-1 <i>H</i> -dibenz( <i>c,f</i> )imidazo(1,5- <i>a</i> )azepine	285.779
Ketotifen fumarate	Zaditor	other	4-(1-Methylpiperidin-4-ylidene)-4,9-dihydro-10 <i>H</i> -benzo[4,5]cyclohepta[1,2- <i>b</i> ]thiophen-10-one	425.5075
Loratadine*	Claritin, Claratyne	other	Ethyl 4-(8-chloro-5,6-dihydro-1 <i>H</i> -benzo[5,6]cyclohepta[1,2- <i>b</i> ]pyridin-1 <i>l</i> -ylidene)-1-piperidinecarboxylate	382.8938
Terfenadine*	Seldane, Triludan, Teldane	other	( <i>RS</i> )-1-(4-tert-butylphenyl)-4-[(4-[hydroxy(diphenyl)methyl]piperidin-1-yl]-butan-1-ol	471.6891
<b>Third generation antihistamine</b>				
Fexofenadine hydrochloride	Allegra, Fexidine, Telfast, Fastofen, Tilfur, Vifas, Telfexo, Allerfexo, Flexofen	other	(±)-4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-α, α-dimethylbenzeneacetic acid	501.6719

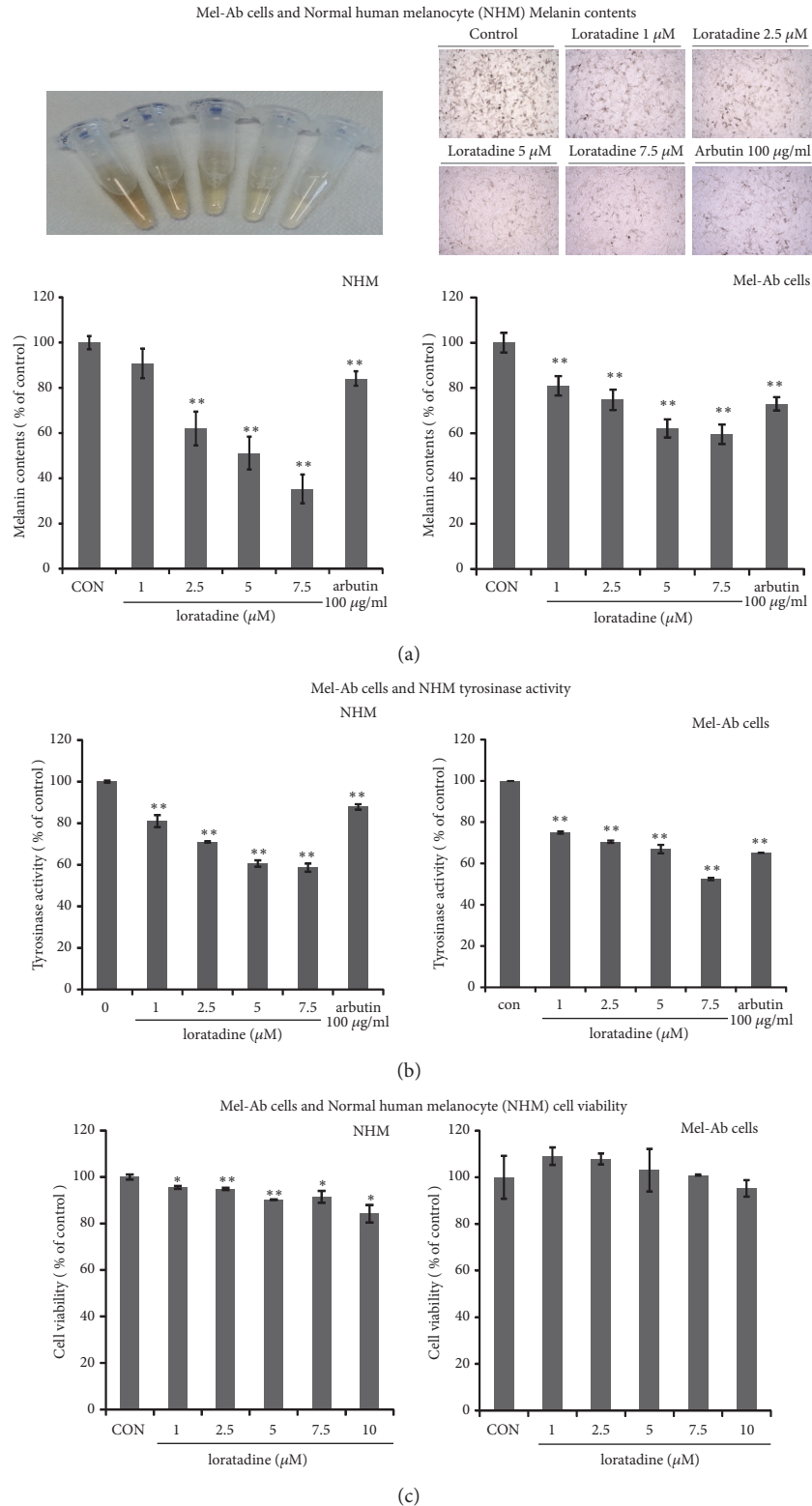


FIGURE 1: Effects of H<sub>1</sub> antihistamine, loratadine, on melanogenesis in normal human melanocytes (NHM) and Mel-Ab cells. (a) NHM and Mel-Ab cells were cultured with 1.0-7.5  $\mu$ M loratadine for 5 days and 4 days each and compared with 100  $\mu$ g/ml arbutin. The cell images were taken using phase contrast microscopy, and the decrease in melanin is evident. (b) Loratadine suppressed tyrosinase activity in NHM and Mel-Ab cells. NHM and Mel-Ab cells were incubated with 1.0-7.5  $\mu$ M loratadine for 5 days and 4 days each, and the cellular tyrosinase activity was then measured. Arbutin (100  $\mu$ g/ml) was used as a positive control. Each determination was made in triplicate, and the data represent the mean  $\pm$  SD. \*p < 0.05 and \*\*p < 0.01 in comparison with the untreated control. (c) The viability of NHM and Mel-Ab cells was not affected by treatment with 1.0-7.5  $\mu$ M loratadine for 24 hr. Cell viability was determined using MTT assay. Each measurement was made in triplicate.

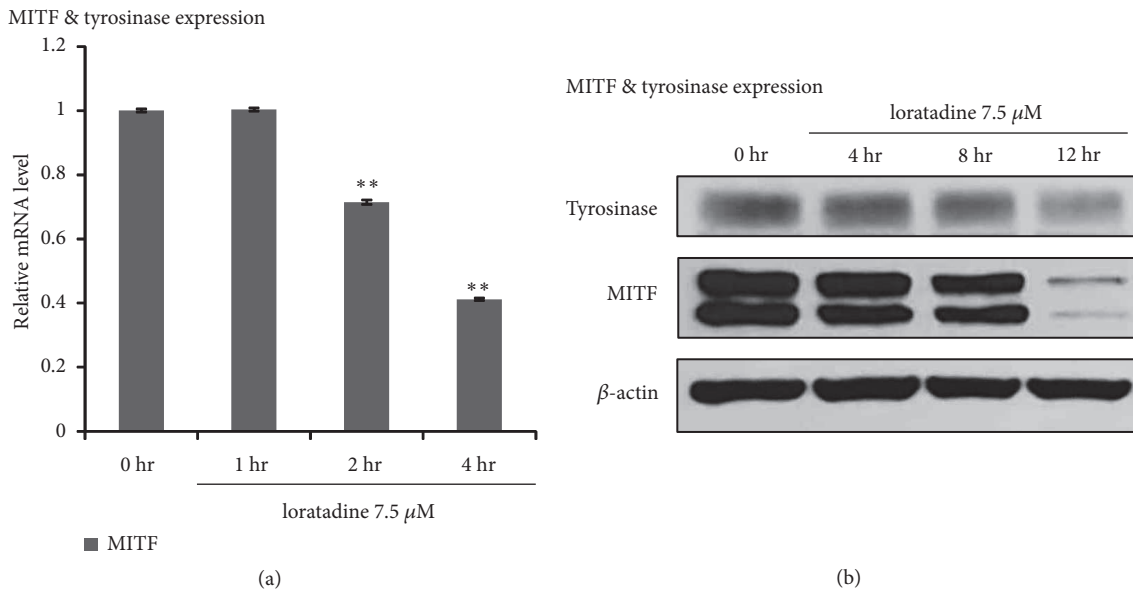


FIGURE 2: Inhibitory effect of loratadine on mRNA and protein expression of MITF and tyrosinase. (a) Loratadine dramatically decreased the mRNA levels of MITF after 4 hr. (b) Loratadine reduced the protein levels of MITF and tyrosinase. NHM were incubated with 7.5  $\mu$ M loratadine at indicated time. Whole-cell lysates were then analyzed by western blotting using antibodies against MITF and tyrosinase. Normalization was achieved by dividing the values for individual bands by the densitometry values for  $\beta$ -actin in the same lane. The value of densitometry was graphed with the mean  $\pm$  SD. \* $p < 0.05$ .

studies about melanogenesis of  $H_1$  antihistamine [6, 15]. For example, mepyramine, an  $H_1$  antihistamine, did not inhibit melanogenesis that is induced by histamine [6]. Therefore, first we screened antimelanogenic effects by  $H_1$  antihistamines using LOPAC chemical library (Table 1). Among them, ebastine, clemisole, terfenadine, and loratadine significantly decreased the melanin content, but loratadine was ultimately selected as its dose-dependent linear hit without affecting cellular viability. Our study found that  $H_1$  antihistamine, especially loratadine, demonstrates obvious antimelanogenic effects in NHM. Loratadine led to the significant inhibition of mRNA and protein expression level of MITF, which in turn suppressed tyrosinase, a key enzyme that controls melanogenesis.

Akt activation has been reported to reduce melanogenesis via transcriptional downregulation of MITF gene expression [16]. Furthermore, in other mechanism, PI3K/Akt/GSK-3 $\beta$  signaling pathway regulates posttranslational modification and proteasomal degradation of MITF protein [17]. In our present study, loratadine suppressed the MITF mRNA expression in NHM, which reversely increased after inhibition of Akt pathway by the selective inhibitor of PI3K, LY294002. Therefore, antimelanogenic effects of loratadine in NHM are shown to be related to activation of PI3K/Akt/GSK-3 $\beta$  signaling and the subsequent decrease in the MITF mRNA level.

Unlike  $H_2$  receptor, which is bound to  $G_{as}$  protein and regulates melanogenesis via cAMP/PKA/CREB signaling pathway,  $H_1$  receptor mainly acts by coupling  $G_{aq/11}$  proteins, which in turn activate inositol trisphosphate

(IP<sub>3</sub>)/diacylglycerol (DAG) pathway and subsequently localizing PKC enzymes to membrane [9]. PKC- $\beta$ II, a regulator of tyrosinase activity, especially, is known to increase melanogenesis and the activity of PKC- $\beta$ II is determined by the membrane localization [18]. As expected, loratadine did not affect the phosphorylation of CREB, but reduced activity of PKC- $\beta$ II.

Our study had several limitations. Although loratadine showed the antimelanogenic effect at the cellular level, these results do not always provide the same outcomes as a clinical manner. Therefore, for practical application of the results, further clinical studies will be required to determine the therapeutic regimen of loratadine for treatment of hyperpigmentary disorders in humans.

Taken together, we demonstrated strong antimelanogenic effect of  $H_1$  antihistamine, loratadine via modulating Akt/MITF, and PKC- $\beta$ II signaling. Considering common use of  $H_1$  antihistamines in dermatologic practice, the antimelanogenic effects of loratadine may have potentially significant and useful roles. Although further clinical studies will be needed, loratadine may give benefits for treatment of hyperpigmentary changes accompanied by various types of dermatitis.

## Data Availability

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

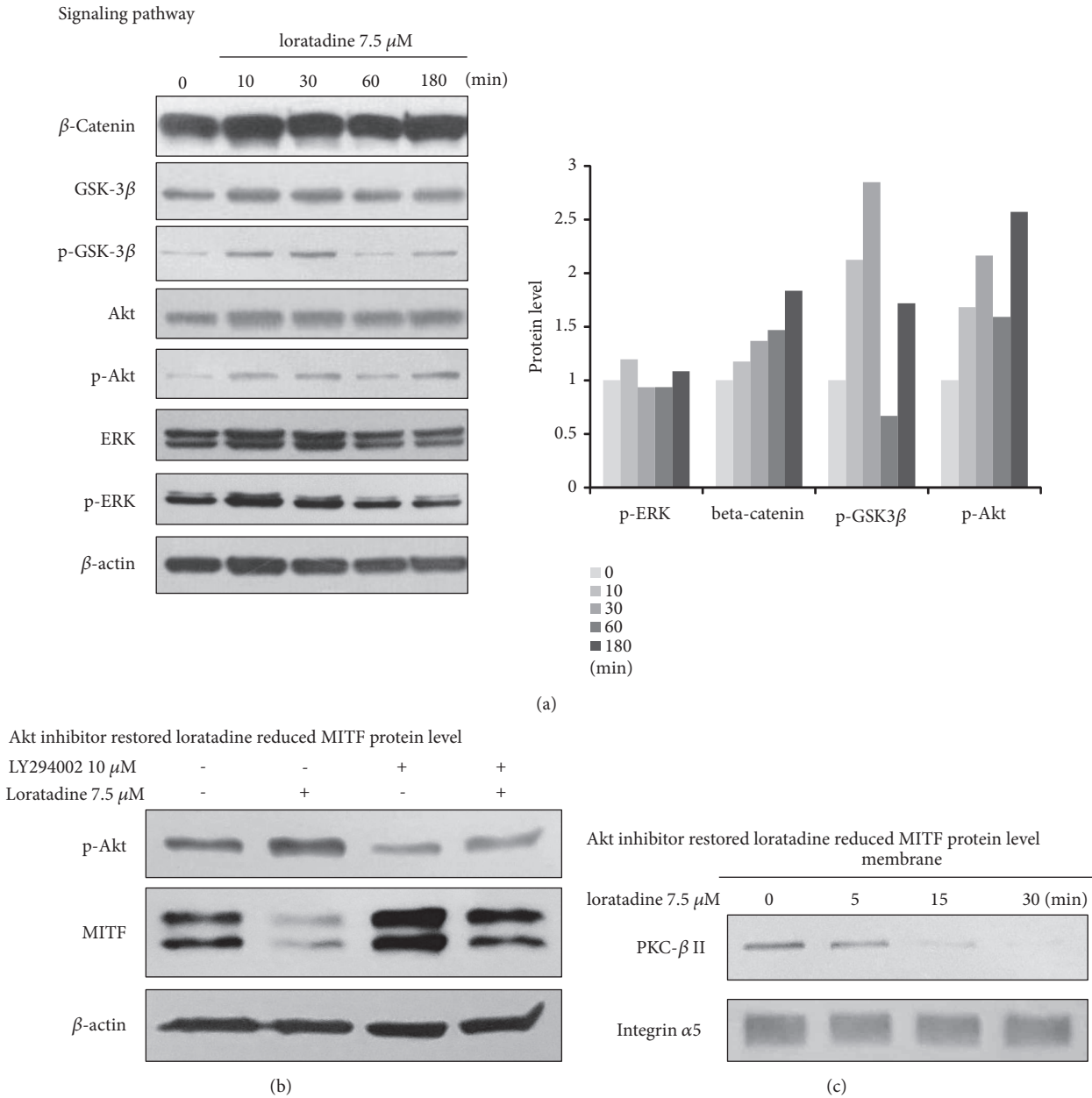


FIGURE 3: The effects of loratadine on melanogenesis-related signaling pathways. After excluding the influence of PMA by 3 hr starvation, NHM were treated with 7.5  $\mu$ M loratadine for the indicated times. (a) Whole-cell lysates were analyzed by western blot using antibodies,  $\beta$ -catenin, phospho-Erk, phospho-Akt, and phospho-GSK-3 $\beta$ , and equal protein loading was confirmed by the  $\beta$ -actin levels. Phospho-Akt and phospho-GSK-3 $\beta$  were consistently elevated by loratadine. (b) LY294002, a selective inhibitor of PI3K, could reverse the increase of phospho-Akt in loratadine treated NHM. (c) Loratadine treatment reduced the amount of PKC- $\beta$ II in the membrane fraction, thereby decreasing its activity.

**Disclosure**

Part of this work has previously been presented at European Society for Pigment Cell Research 2016 Meeting (Poster session) and the Ninth Korean Society for Investigative Dermatology Research Camp 2017 (Free communications II).

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Supplementary Materials**

Supplementary Figure 1: effects of H1-receptor agonist and antagonists on melanogenesis. Supplementary Figure 2:

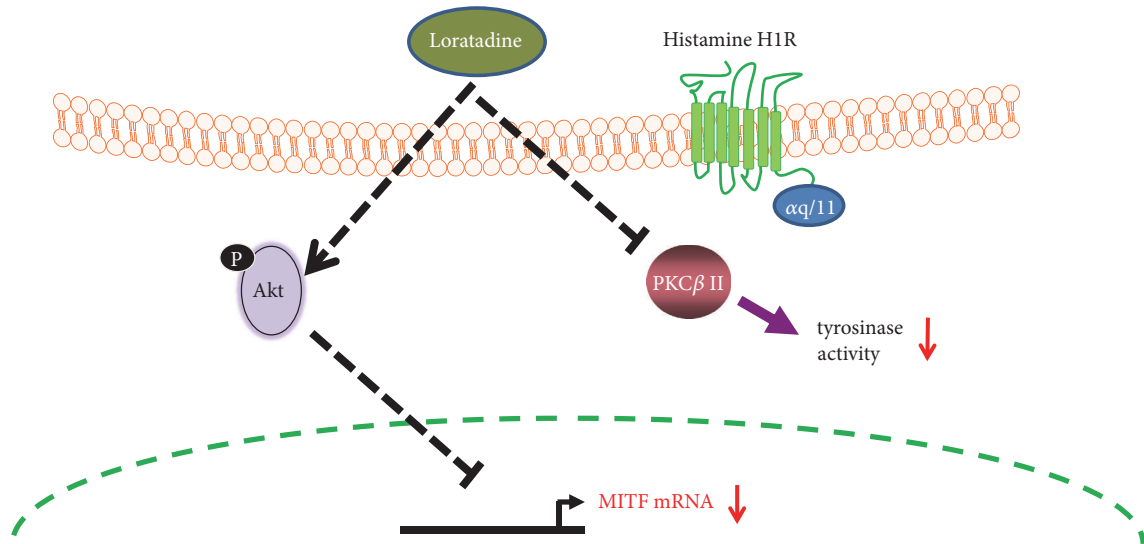


FIGURE 4: Antimelanogenic effect of H<sub>1</sub> antihistamine, loratadine via modulating Akt/MITF, and PKC-βII signaling.

effects of loratadine on cellular viability in normal human melanocytes. (*Supplementary Materials*)

## References

- [1] T. K. Y. Noh, S. J. O. Choi, B. Y. O. Chung et al., "Inflammatory features of melasma lesions in Asian skin," *The Journal of Dermatology*, vol. 41, no. 9, pp. 788–794, 2014.
- [2] H.-R. Moon, Y. J. Kim, J. M. Jung et al., "Acquired bilateral telangiectasia macularis eruptiva perstans: a unique clinical feature of photodamaging rather than a subtype of cutaneous mastocytosis," *The Journal of Dermatology*, vol. 44, no. 11, pp. 1276–1280, 2017.
- [3] M. W. Lassalle, S. Igarashi, M. Sasaki, K. Wakamatsu, S. Ito, and T. Horikoshi, "Effects of melanogenesis-inducing nitric oxide and histamine on the production of eumelanin and pheomelanin in cultured human melanocytes," *Pigment Cell Research*, vol. 16, no. 1, pp. 81–84, 2003.
- [4] C. H. Van Niekerk and A. E. M. Prinsloo, "Effect of skin pigmentation on the response to intradermal histamine," *International Archives of Allergy and Immunology*, vol. 76, no. 1, pp. 73–75, 1985.
- [5] Y. Tomita, K. Maeda, and H. Tagami, "Stimulatory effect of histamine on normal human melanocytes in vitro," *The Tohoku Journal of Experimental Medicine*, vol. 155, no. 2, pp. 209–210, 1988.
- [6] M. Yoshida, Y. Takahashi, and S. Inoue, "Histamine induces melanogenesis and morphologic changes by protein kinase A activation via H<sub>2</sub> receptors in human normal melanocytes," *Journal of Investigative Dermatology*, vol. 114, no. 2, pp. 334–342, 2000.
- [7] W. J. Lee, S. Y. Jo, M. H. Lee et al., "The effect of MCP-1/CCR2 on the proliferation and senescence of epidermal constituent cells in solar lentigo," *International Journal of Molecular Sciences*, vol. 17, no. 6, 2016.
- [8] E.-H. Kim, M.-K. Kim, H.-Y. Yun et al., "Menadione (vitamin K<sub>3</sub>) decreases melanin synthesis through ERK activation in Mel-Ab cells," *European Journal of Pharmacology*, vol. 718, no. 1–3, pp. 299–304, 2013.
- [9] S. J. Hill, C. R. Ganellin, H. Timmerman et al., "International union of pharmacology. XIII. Classification of histamine receptors," *Pharmacological Reviews*, vol. 49, no. 3, pp. 253–278, 1997.
- [10] F. E. R. Simons, "Advances in H<sub>1</sub>-antihistamines," *The New England Journal of Medicine*, vol. 351, no. 21, pp. 2203–2217, 2004.
- [11] J. L. Reynolds, J. Akhter, and D. L. Morris, "In vitro effect of histamine and histamine H<sub>1</sub> and H<sub>2</sub> receptor antagonists on cellular proliferation of human malignant melanoma cell lines," *Melanoma Research*, vol. 6, no. 2, pp. 95–99, 1996.
- [12] N.-H. Kim and A.-Y. Lee, "Histamine effect on melanocyte proliferation and vitiliginous keratinocyte survival," *Experimental Dermatology*, vol. 19, no. 12, pp. 1073–1079, 2010.
- [13] H. J. Lee, M. K. Park, E. J. Lee et al., "Histamine receptor 2-mediated growth-differentiation factor-15 expression is involved in histamine-induced melanogenesis," *The International Journal of Biochemistry & Cell Biology*, vol. 44, no. 12, pp. 2124–2128, 2012.
- [14] T. Anbar, W. Westerhof, N. Badawy et al., "The effect of H<sub>1</sub> and H<sub>2</sub> receptor antagonists on melanogenesis," *Indian Journal of Dermatology, Venereology and Leprology*, vol. 78, no. 1, pp. 64–67, 2012.
- [15] M. T. McEwan and P. G. Parsons, "Regulation of tyrosinase expression and activity in human melanoma cells via histamine receptors," *Journal of Investigative Dermatology*, vol. 97, no. 5, pp. 868–873, 1991.
- [16] M. Khaled, L. Larribere, K. Bille, J.-P. Ortonne, R. Ballotti, and C. Bertolotto, "Microphthalmia associated transcription factor is a target of the phosphatidylinositol-3-kinase pathway," *Journal of Investigative Dermatology*, vol. 121, no. 4, pp. 831–836, 2003.
- [17] H.-C. Huang, S.-J. Chang, C.-Y. Wu, H.-J. Ke, and T.-M. Chang, "[6]-Shogaol inhibits α-MSH-induced melanogenesis through the acceleration of ERK and PI3K/Akt-mediated MITF degradation," *BioMed Research International*, vol. 2014, Article ID 842569, 9 pages, 2014.
- [18] H. Jung, H. Chung, S. E. Chang et al., "Syndecan-2 regulates melanin synthesis via protein kinase C βII-mediated tyrosinase activation," *Pigment Cell & Melanoma Research*, vol. 27, no. 3, pp. 387–397, 2014.