Fenofibrate suppresses melanogenesis in B16-F10 melanoma cells via activation of the p38 mitogen-activated protein kinase pathway

Yu-Chun Huang, Kao-Chih Liu, Yi-Ling Chiou, Chao-Hsun Yang, Tien-Hui Chen, Ting-Ting Li, Li-Ling Liu

1. Introduction

The color of mammalian skin and hair is determined by a number of factors, including the distribution of the pigment melanin. Melanocytes, which synthesize melanin, act as pivotal regulators of skin pigmentation, or melanogenesis. Of the five members of melanocortin receptors, the melanocortin 1 receptor (MC1R), expressed primarily in melanocytes, plays a critical role in regulation of pigmentation [1]. Microphthalmia-associated transcription factor (MITF) is the most critical transcription factor in the regulation of the development and differentiation of melanocytes. Transcription dependent on MITF regulates the expression of tyrosinase and tyrosinase-related proteins that control the conversion of tyrosine to melanin [2]. The MITF promoter is regulated in part by various other transcription factors, such as paired box protein PAX3, SRY-related HMG-box 10, Lymphoid enhancer-binding factor 1/T cell-specific factor, and cAMP response element-binding protein [1]. Accordingly, multiple signaling pathways are integrated into the modulation of melanocyte functions. Endogenous and exogenous factors up-regulate or down-regulate melanogenic processes, and dysregulation of these factors causes pigmentation disorders [3].

Biochemical reaction and exposure to environmental stress such as ultraviolet radiation and pollutants can generate reactive oxygen species (ROS), including oxygen radicals, and certain nonradicals that are either oxidizing agents and/or are easily converted into radicals [4,5]. Accumulation of ROS has been shown in the epidermis of vitiligo patients [6]. Gene expression pattern is regulated by ROS via modulation of transcription factor activity particularly nuclear factor kappa B (NF-kB), activator protein-1 (AP-1), and peroxisome proliferator-activated receptor (PPAR) family of transcriptional activators. Oxidative stress is defined as an imbalance between cellular production of oxidant molecules and the availability of appropriate antioxidants that defend against them [7]. The reduction–oxidation (redox) balance plays an important role in physiological modulation. Continued oxidative stress can lead to the development of chronic diseases, including cancer, neurodegeneration, cardiovascular, and metabolic diseases.
Obesity is associated with insulin resistance and type 2 diabetes mellitus (DM). Obese and DM patients have many cutaneous problems, which may be associated with prolonged wound healing, inflammatory dermatoses, and melanoma. A link between excess adiposity and malignant melanoma has been found by various epidemiological studies [8,9]. Therapeutic agents, such as fibrates and thiazolidinediones, have been evaluated for the treatment of patients with type 2 DM with multiple cardiometabolic risk factors [10]. Moreover, a meta-analysis of trials involving statins, fibrates, and melanoma risk was performed [11]. Fibrates (fenofibrate, gemfibrozil) activate PPARα. In clinical trials, significantly fewer patients treated with gemfibrozil were diagnosed with melanoma compared with the control group [12]. Thiazolidinediones, also known as glitazones, are potent and highly selective agonists for PPARγ.

Three PPAR isotypes have been isolated – PPARα, PPARβ/δ, and PPARδ – with distinct tissue distributions and biological activities. Each PPAR heterodimerizes with the retinoid X receptor to form a complex that translocates to the nucleus and regulates gene expression. PPARs are involved in various skin diseases, particularly those involving inflammation, epidermal hyperproliferation, and differentiation [13–15]. PPARα and PPARγ agonists exhibit the potential therapeutic application for the treatment of pigmentary disorders such as melasma, vitiligo, and postinflammatory hyperpigmentation [16]. For instance, diocid acid, through binding of PPARγ, exhibited lightening effects in the treatment of melasma [17]. The mRNA of all three PPAR subtypes is expressed in melanocytes [16]. Cultured mouse and human melanoma cells produce more PPARα and PPARγ protein compared to melanocytes [18]. PPARα and PPARγ are expressed in B16-F10 and B16 cells, respectively [18,19]. Nevertheless, the molecular events following PPAR ligand binding have not been well investigated in melanocytes. Therefore, the melanogenic activity of murine B16-F10 melanoma cells treated with various PPAR agonists was assessed in the present study.

2. Materials and methods

2.1. Chemicals and reagents

Fenofibrate, GW0742, 3-[4S]-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), L-3,4-dihydroxyphenyalanine (L-DOPA), N-acetyl-L-cysteine (NAC), and melanin were obtained from Sigma–Aldrich (St. Louis, MO, USA). Cigitazone, MG132, SB203580, and T0901317 were purchased from Enzo Life Sciences (Farmingdale, NY, USA). Fenofric acid was purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). GW6471 was purchased from Tocris Bioscience (Bristol, UK). GW7647 was purchased from Cayman Chemical (Ann Arbor, MI, USA). An antibody against tyrosinase was obtained from Epitomics (Burlingame, CA, USA). The anti-MIF antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against p38 MAPK and phosphorylated p38 MAPK (Thr180/Tyr182) were purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies against α-tubulin and actin were supplied by Millipore (Temecula, CA, USA).

Fenofibrate, fenofric acid, GW0742, GW 6471, GW 7647, cigitazone, MG132, T0901317, and SB203580 were dissolved in dimethylsulfoxide (DMSO) and further diluted in culture medium. To characterize the action mechanism of fenofibrate, B16-F10 cells were pretreated with MG132, T0901317, SB203580 or NAC for 30 min and then treated with fenofibrate for further times. The final DMSO concentration in the medium was 0.1% and did not affect cellular function or the assay systems used in this study.

2.2. Cell culture

The murine B16-F10 melanoma cell line was purchased from the Bioresource Collection and Research Center (Hsinchu, Taiwan), and cells were maintained in Dulbecco’s modified Eagle’s medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Biological Industries, Kibbutz Beit Haemek, Israel) in a humidified incubator at 37 °C with 5% CO2.

2.3. Cell viability

Cell viability was determined using the MTT assay. Briefly, the B16-F10 cells were seeded at a density of 5 × 10^3 cells/well on 96-well plates and cultured overnight as described above. The medium was replaced with fresh medium containing test compounds at various concentrations. After incubation for 48 h at 37 °C with 5% CO2, MTT was added to a final concentration of 0.5 mg/mL, and the cells were incubated at 37 °C for 2 h. Finally, the cells were lysed, and absorbance was detected at 550 nm. The number of cells was determined by interpolation of the detected absorbance density values using a standard correlation between known cell numbers and their absorbance density values.

2.4. Melanin content determination

Melanin content was measured using a previously described method [20]. Briefly, cells were seeded at a density of 2 × 10^5 cells/60-mm culture dishes and cultured as described above. After overnight incubation, the cells were cultured with or without test compounds for 48 h. The medium was removed, and the cells were washed twice with phosphate-buffered saline (PBS) and harvested by trypsinization using 0.05% trypsin/0.02% EDTA. The harvested cells were centrifuged, the pellet was dissolved by adding 1 N NaOH, and the mixture was incubated at 60 °C for 1 h. The amount of melanin in the solution was determined by measuring the absorbance at 470 nm using the microplate reader (BioTek Instruments, Winooski, VT, USA). The total melanin content was estimated using a standard curve of synthetic melanin. The melanin content was calculated by normalizing the melanin contents to total cellular protein (μg of melanin/mg of protein) and reported as a percentage of control. The protein content was determined using the Micro BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA).

2.5. DOPA-staining assay (tyrosinase zymography)

A DOPA-staining assay was performed as described by Sato and co-workers [21] with slight modifications. Briefly, the cells were lysed in 0.1 M sodium phosphate buffer (pH 6.8) containing 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride and aliquots of 30 μg of normalized proteins were resolved by 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) under non-denaturing conditions. Afterward, the gels were transferred into a staining solution containing 1 mM L-DOPA and incubated in the dark for 1 h at 37 °C. Tyrosinase activity was visualized in the gels as dark, melanin-containing bands. To directly assess the activity of tyrosinase, fenofibrate was added to the untreated cell lysate and incubated for 5 min at room temperature. The cell lysates were mixed with L-DOPA solution and incubated at 37 °C for 2 h, as described above.

2.6. Western blots

Whole cell lysates were prepared using RIPA buffer. Aliquots of the cell lysates (20 μg each lane) were separated by SDS–PAGE, transferred onto polyvinylidene difluoride membranes, and blotted.
with the indicated antibodies: anti-tyrosinase 1:100, anti-MITF 1:500, anti-P-p38 1:5000, anti-p38 1:6000, anti-Bcl-2 1:100, anti-actin 1:20,000, anti-α-tubulin 1:20,000, and anti-GAPDH 1:5000. Horseradish peroxide-conjugated goat anti-rabbit and anti-mouse immunoglobulin (Jackson Immunoresearch Laboratories Inc., West Grove, PA, USA) were used at 1:12,000 and 1:5000, respectively. The proteins were detected using an enhanced chemiluminescence kit (Amersham Biosciences, Buckinghamshire, UK). Western blots were representative of at least three independent experiments. Quantitative analysis of specific bands was performed using Image Quant analysis software (GE Healthcare).

2.7. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted from cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA), and 1 μg of total RNA was reverse transcribed to cDNA using the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). The real-time PCR amplifications were performed in a MiniOpticon™ real-time PCR system using iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA) and pre-designed gene specific primer sets. Primers for mouse genes were as follows: Tyrosinase, forward 5′-AACAGCGATGGAAACTA-3′, reverse 5′-AACAGGTTGAGTCTC-3′; MITF, forward 5′-TCGGTITAGAGTCCTCAA-3′, reverse 5′-AAAGTCAGAAGGTATT-3′; Melanocortin 1 receptor (MC1R), forward 5′-TCCTCTATGTGACAAGAA-3′, reverse 5′-ATTCTAGGCTCTGTC-3′; Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward 5′-TCGAGGATACAGAAAC-3′, reverse 5′-GAATTCGTGAGAGATG-3′. The expression of GAPDH was used as the internal standard. PCRs were done in triplicates. Genes of interest from three separate experiments were analyzed by CFX manager software (version 2.1), and expressed as fold changes over that in control group.

2.8. Statistics

The data were expressed as the means ± standard error of the mean (S.E.M.) of the indicated number of separate experiments. A one-way analysis of variance was performed for multiple comparisons. In the event of significant variation among treatment groups, the mean values were compared to the respective control using Dunnett’s test. P-values less than 0.05 were considered statistically significant.

3. Results

3.1. The PPARα agonist fenofibrate decreases melanin synthesis

Briefly, B16-F10 melanoma cells were treated with PPAR agonists for 48 h to assess the effect of the agonists on cell viability. Fenofibrate, GW0742, and ciglitazone are agonists of PPARα, β/δ, and γ, respectively. The MTT assay revealed that the compounds did not alter cell survival even at 10 μM (Fig. 1A). The cellular melanin contents were evaluated simultaneously. As shown in Fig. 1B, fenofibrate, but not the other PPAR agonists, decreased the melanin content after 24 or 48 h of incubation. The inhibitory effect of fenofibrate on melanin synthesis was time- and concentration-dependent (Fig. 1B and Fig. 2A). Melanin production was inhibited by approximately 40% in cells treated with 10 μM fenofibrate for 48 h. Although the inhibitory effect of 20 μM fenofibrate was more potent than that of 10 μM (Fig. 2A), an anti-proliferative effect, as noted in a previous study [19], was also observed at the higher concentration. We also evaluated the effects of fenofibrate on cell proliferation and membrane integrity using a trypan blue exclusion assay, and no statistically significant differences were observed between 10 μM fenofibrate-treated cells and DMSO-treated control cells (data not shown). The cell number of 20 μM fenofibrate-treated cells was reduced to 54.6 ± 9.4% of the control cell counts (P < 0.001). Therefore, 10 μM fenofibrate was used for subsequent experiments.

3.2. PPARα does not mediate the effect of fenofibrate on melanogenic activity

To further elucidate the mechanism of fenofibrate on melanin production, we sought to determine if the effects of fenofibrate are mediated through the activation of the PPARα receptor. We therefore examined the biological effects of other potent PPARα agonists, including a fibric acid derivative and GW7647. Fenofibrate was metabolized to the active metabolite fenofibric acid by esterase following oral administration. Surprisingly, fenofibric acid failed to diminish the melanin content at every concentration tested (Fig. 2A), including 100 μM, the highest concentration that was studied (data not shown). The results shown in Fig. 2B indicate that the addition of 10 μM GW7647 alone did not significantly decrease melanogenesis. We also examined the effect of GW6471, a specific inhibitor of the DNA binding of PPARα [22], on melanin levels. The suppression of melanin levels by fenofibrate was not altered by the GW6471 pretreatment. These results imply that the PPARα antagonist did not affect the fenofibrate-repressed melanogenic activity. Interestingly, the liver X receptor (LXR)
agonist, T0901317, could override the fenofibrate-induced repression of melanogenesis (Fig. 2C). Thus, the biological effects of fenofibrate do not seem to be dependent on the transcriptional activity of PPARγ. We speculate that intrinsic LXR signaling might contribute to melanogenesis in B16-F10 cells.

3.3. Fenofibrate inhibits tyrosinase activity

Intracellular tyrosinase activity was confirmed by the DOPA-staining assay (Fig. 3). After densitometric analysis, we observed that 10 μM fenofibrate significantly reduced tyrosinase activity to approximately 54.6 ± 0.06% of that of the control. To exclude the possibility of a direct influence of fenofibrate on tyrosinase activity, we added fenofibrate directly to an untreated cell lysate and measured the in vitro tyrosinase activity (data not shown). Fenofibrate did not directly regulate tyrosinase activity in the in vitro system, but did inhibit intracellular tyrosinase activity in B16-F10 cells.

3.4. Fenofibrate influences signaling pathways that mediate melanogenesis

The expression of melanogenesis-related proteins is crucial to the synthesis of pigments and is modulated by transcription factors. Therefore, the cell lysates that were obtained after fenofibrate treatment were subjected to western blot analysis. We found that the protein expression of tyrosinase and MITF was repressed with increasing concentrations of fenofibrate (Fig. 4). Quantification of the detected signal revealed that the protein levels of tyrosinase and MITF following treatment with 10 μM fenofibrate were reduced to 0.52 ± 0.08- and 0.73 ± 0.05-fold ($P < 0.05$, $n = 3$) of the DMSO-treated control, respectively.

Among the signal transduction pathways related to pigmentation, the p38 mitogen-activated protein kinase (MAPK) pathway is involved in the regulation of melanogenesis [23,24]. To further characterize fenofibrate’s mechanism of action, we performed a time-course study using 10 μM fenofibrate. Fig. 5A shows that fenofibrate induced early phosphorylation of p38 MAPK within 1 h, and this phenomenon was sustained until 3 h. The phosphorylation of p38 MAPK persistent elevated even at 12- and 24-h of fenofibrate treatment.

p38 MAPK might control tyrosinase stability by regulating its proteasome-dependent degradation [24]. Therefore, we used MG132, a membrane-permeable proteasome inhibitor, to assess whether fenofibrate-dependent suppression of tyrosinase expression is involved in the regulation of the ubiquitin–proteasome pathway. Accordingly, we observed that the fenofibrate-induced decrease of tyrosinase expression was prevented by MG132 pretreatment (Fig. 5B). To confirm the involvement of p38 MAPK signaling, the inhibitor SB203580 was used to assess how gene expression was affected by fenofibrate. We examined the mRNA...
expression of tyrosinase and MITF. Quantitative RT-PCR analysis revealed that fenofibrate treatment slightly affected gene expression of tyrosinase and MITF, about 0.70 ± 0.11- and 0.77 ± 0.15-fold decrease, respectively. We further evaluated the expression level of MC1R. The results revealed that expression of MC1R mRNA was significantly inhibited by fenofibrate after a 3-h treatment (Fig. 5C). However, the gene expression influenced by fenofibrate was not significantly changed in the presence of SB203580. We also determined the effect of SB203580 on fenofibrate-decreased melanin synthesis. According to Fig. 5D, the melanin count suppressed by fenofibrate was approximately reverted to the control level in the presence of SB203580. These results demonstrate that the activation of p38 MAPK signaling contributes to fenofibrate-mediated suppression of melanogenesis.

To examine the possible roles of oxidative stress on fenofibrate-mediated inhibitory effects of melanogenesis, the ROS scavenger, N-acetyl-L-cysteine (NAC), was accompanied with fenofibrate treatment. The results of melanin synthesis assay (Fig. 5D) displayed that fenofibrate-decreased melanin contents was not counteracted by the NAC pretreatment. It seems that the intracellular ROS generation is not involved in the inhibitory effect of fenofibrate on melanogenesis.

Since the early phase of apoptosis may obscure the effects of fenofibrate on melanogenesis, the expression of Bcl-2 family proteins were examined by the Western blots. Fig. 5E showed that the protein levels of Bax and Bcl-2 remained unchanged at any time points of fenofibrate treatment. These results clarify that the effects of fenofibrate on the regulation of melanogenesis is not related to the apoptotic events.

4. Discussion

PPARs and their corresponding ligands have been shown to regulate important physiological functions in the body. In human melanocytes, activators of PPARγ (ciglitazone) appear to stimulate melanogenesis by increasing tyrosinase activity rather than tyrosinase expression [25]. Grabacka et al. [19] showed that mouse B16-F10 cells express PPARα and that the transcriptional activation of PPARα by fenofibrate inhibits migration and anchorage-independent growth. Further work in mouse melanoma S91 cells indicated that activation of PPARγ induces events resembling differentiation. Exposure to ciglitazone regulates MITF in a biphasic manner and induces tyrosinase activity [26]. In contrast with other PPARβ/δ or PPAR γ agonists, we showed that fenofibrate alone inhibited melanogenesis in B16-F10 cells. The decreased levels of melanin were accompanied by a parallel decline in tyrosinase activity. Comparison of the phenomena observed in the present study with those observed in previous research indicates that PPAR agonists affect different regulatory pathways.

Fenofibrate has long been thought to act by binding to PPARα to achieve the therapeutic lowering of lipid levels. At a higher concentration (25 μM), fenofibrate showed a twofold stimulation of the PPAR responsive elements in B16-F10 cells [19]. According to the data shown here, fenofibrate diminishes melanin levels, and this phenomenon was not reversed by the selective PPARα antagonist GW6471. A high affinity compound for PPARα, GW7647, also did not alter melanin synthesis. In addition, we observed that the major active metabolite of fenofibrate, fenofibric acid, failed to inhibit melanin production. Fenofibric acid contains a carboxylic acid moiety instead of an isopropyl ester moiety in fenofibrate. The structural analysis reveals that the carboxyl group aligns on the same side as the ketone carbonyl group in fenofibric acid. However, the carboxyl group of fenofibrate is located away from the ketone carbonyl group [27]. It can be inferred that the different orientation of functional groups in fenofibrate and fenofibric acid may be related to the distinct effects of such chemicals interact with their target molecules in melanoma cells. Nevertheless, the affinity of the ligands for each PPAR subtypes varies [16]. Recent evidence has shown that unmetabolized fenofibrate results in different enzymatic activity than fenofibric acid [28]. A single PPAR ligand may interact with more than one receptor subtype. It has also been proposed that fenofibric acid is a PPARγ agonist, while fenofibrate is a LXR agonist [29]. The fibrates display greater affinity for LXRs than the corresponding fibric acids do for PPARα [30]. The LXR agonist T0901317 restored the fenofibrate-induced suppression of melanogenesis. The direct effects of fenofibrate on PPARα can be excluded because fenofibric acid and GW7647, which have higher affinities for PPARα than fenofibrate, failed to decrease melanin synthesis in B16-F10 cells. Therefore, we assume that fenofibrate suppressed melanin synthesis via a PPAR-independent mechanism. Similarly, fenofibrate suppresses the growth of human hepatocellular carcinoma cells and induces apoptosis in mantle lymphoma by a PPARα-independent mechanism [31,32]. A previous report noted that the expression of the nuclear receptor LXRα in perilesional human melanocytes is higher relative to its expression in the normal skin of a vitiligo patient [33]. It can be speculated that LXRs are involved in the fenofibrate-mediated regulation of melanin production. The B16-F10 melanoma cell line with altered pigmentation signaling pathways is a well-established model for the study of melanocyte biology [34]. Further work to assess the effects of fenofibrate on normal human melanocytes or reconstituted human epidermis will clarify the physiological relevance of fenofibrate.

Tyrosinase is the rate-limiting enzyme for melanogenesis, and its stability is regulated by endoplasmic reticulum-associated protein degradation or the endosomal/lysosomal degradation system [35]. Anisomycin, a well-characterized and relatively specific activator of p38 MAPK, causes tyrosinase degradation in B16-F0 melanoma cells and in normal human melanocytes. In addition,
that degradation is attenuated by MG132 [24]. In the present study, fenofibrate exhibited effects similar to those of anisomycin. The results suggest that fenofibrate regulates the melanogenesis through the post-translation modulation. Since fenofibrate also affected the mRNA levels of tyrosinase, MITF, and MC1R, these data do not obviate involvement of transcriptional regulation in the fenofibrate-decreased tyrosinase expression. However, SB203580 could not significantly restore the fenofibrate-induced decrease of gene expression. It has been demonstrated that p38 silencing showed no significant change in MITF mRNA transcription [24]. Thus, our data on the effects of fenofibrate on the melanogenic enzymes is partially consistent with the previous study. Recently, SB203580, a pyridinyl imidazole compound that specifically inhibits p38α and p38β [36], has been shown to inhibit melanogenesis in a p38-independent manner [24,37]. It seems that the pyridinyl imidazole class of compounds employed to study p38 MAPK-dependent melanogenesis is inadequate. Further studies using small interfering RNA experiments will address the targets of fenofibrate and resolve the inadequacies of chemical agents.

Western blots illustrated that fenofibrate down-regulated the protein expression of tyrosinase and MITF while up-regulating the phosphorylation of p38 MAPK. Fenofibrate-induced inhibition of melanin synthesis was restored by SB203580. B16-F10 cells expressing MC1R inhibited activation of p38 MAPK, but not ERK and JNK/SAPK, whereas B16-F10 cells expressing siRNA targeting MC1R showed enhanced phosphorylation of p38 MAPK [38]. It is possible that fenofibrate through the perturbation of MC1R expression to regulate p38 MAPK signaling in B16-F10 cells. Taken together, these data suggest that fenofibrate suppresses melanogenesis by the down-regulation of MC1R, activation of the p38 MAPK signaling pathway, and the repression of the MITF signaling pathway. Curcumin, through activation of phosphatidylinositol 3-kinase (PI3K/Akt), extracellular signal-regulated kinase (ERK), or p38 MAPK signaling, inhibits melanogenesis in human melanocytes [39]. During a 48 h incubation, 25 μM fenofibrate significantly interferes with Akt and ERK1/2 signaling pathways in B16-F10 cells [19]. In the present study, we did not explore the interactions of 10 μM fenofibrate with these signaling molecules. Further studies are needed to verify the relationships of fenofibrate with these kinases.

Cellular redox may be key to oxidant-induced activation of the stress-activated protein kinase (SAPK) pathways [40]. p38 MAPK is well accepted as a stress-stimulated MAPK and activated by diverse stressors, such as ROS generation. Hydrogen peroxide...
(H₂O₂) concentration in the mM range has been shown to increase p38 MAPK activity in B16-F10 cells [38]. Based on the results of Fig. 5D, fenofibrate-decreased melanin synthesis was not efficiently offset by NAC, a H₂O₂ scavenger, whereas SB203580 did it. Besides, treatment of fenofibrate did not show stimulatory effect on ROS generation as detected by 2',7'-dichlorofluorescein diacetate with flow cytometry analysis (data not shown). It is likely that the inhibitory effect of fenofibrate on melanogenesis is not relevant to the intracellular ROS generation. Animal studies demonstrated that fenofibrate through PPARα activation significantly attenuated oxidative damage in brain by modulating antioxidant defense mechanisms, such as level of reduced glutathione and enzyme activity of catalase and superoxide dismutase [41]. Further elucidating mode of antioxidant activity will help to refine the redox effects of fenofibrate. A recent study showed that fenofibrate elicits dual beneficial effects in retinal pigment epithelium by down-regulation of stress-mediating signaling and induction of autophagy and survival pathways [42]. Fenofibrate triggers Bim-mediated apoptosis of glioblastoma cells in vitro [43]. We demonstrated that protein expression of Bcl-2 and Bax was not altered by the fenofibrate. The effect of fenofibrate on melanogenesis is independent of apoptosis.

Thiazolidinediones are a class of molecules that are chemically related to fibrates [44]. Mitochondrial dysfunction induced by fibrates and thiazolidinediones was response to the insulin-sensitizing effects of certain drugs [44,45]. Fenofibrate stimulated the phosphorylation of p38 MAPK and then inhibited the melanin synthesis. Sphingosylphosphorylcholine-induced hypoglycemia is a well-recognized side effect of certain PPARs [46]. It is valuable to consider the role of protein phosphatase on the modulation of melanogenesis. Due to the limited information, whether fenofibrate or cigitazone regulates some protein phosphatases to influence the activation of p38 MAPK pathways is unclear.

5. Conclusions

In summary, we have demonstrated that fenofibrate suppresses melanogenesis through the down-regulation of the DOPA-staining activity and expression of tyrosinase as well as the down-regulation of MITF expression. To the best of our knowledge, this is the first time that fenofibrate has been shown to inhibit melanogenesis by down-regulating MC1R gene expression and activating the p38 MAPK signaling pathway in a manner independent of PPAR activation. The potential to influence skin cancer risks is relevant to the therapeutic use of fenofibrate in conventional disease treatment.

Acknowledgements

This study was supported by Grants from the National Science Council of Republic of China (NSC98-2220-B-126-001-MY3) and Chang Bing Show Chwan Memorial Hospital (CBSH10010010).

References

[14] M. Schmutz, Y.J. Jiang, S. Dubrac, P.M. Elias, K.R. Feinigold, Thiazolidinediones are a class of molecules that are chemically related to fibrates [44]. Mitochondrial dysfunction induced by fibrates and thiazolidinediones was response to the insulin-sensitizing effects of certain drugs [44,45]. Fenofibrate stimulated the phosphorylation of p38 MAPK and then inhibited the melanin synthesis. However, cigitazone did not reveal the negative regulation of melanogenesis. Sphingosylphosphorylcholine-induced hypoglycemia is a well-recognized side effect of certain PPARs [46]. It is valuable to consider the role of protein phosphatase on the modulation of melanogenesis. Due to the limited information, whether fenofibrate or cigitazone regulates some protein phosphatases to influence the activation of p38 MAPK pathways is unclear.


