Baicalein inhibits lipid accumulation by regulating early adipogenesis and m-TOR signaling

Min-Jung Seo 1, Hyeon-Son Choi 1, Hui-Jeon Jeon, Mi-Seon Woo, Boo-Yong Lee *

Department of Food Science and Biotechnology, CHA University, Kyonggi 463-836, South Korea

Abstract

Baicalein is a type of flavonoid that originates from Scutellaria baicalensis. In this study, we examined how baicalein inhibits lipid accumulation during adipogenesis in 3T3-L1 cells. Our data show that baicalein inhibited lipid accumulation during adipogenesis in a dose-dependent manner. Baicalein inhibition was limited to the early adipogenic stage. Cell cycle analysis showed that baicalein induced cell cycle arrest in the G0/G1 phase through cyclin downregulation. In addition, baicalein suppressed the mRNA expression of early adipogenic factors leading to downregulation of late adipogenic factors at mRNA and protein levels. Inhibition of adipogenic factors by baicalein was correlated with downregulation of lipid synthetic enzymes. Additionally, baicalein negatively regulated the m-TOR signaling pathway involved in lipid accumulation during adipogenesis, thus inhibiting phosphorylation of m-TOR and p70S6 K. In a zebrafish study, baicalein significantly reduced lipid accumulation in Nile Red staining. Consistent with a report using cell lines, mRNA expression of adipogenic factors was decreased in a dose-dependent manner by baicalein. This result reflects a reduction in total triglyceride levels based on a triglyceride assay. Our data suggest that baicalein inhibits lipid accumulation by controlling the cell cycle and m-TOR signaling in 3T3-L1 cells, and its anti-adipogenic effect was found in a zebrafish model.

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1. Introduction

Baicalein (5,6,7-trihydroxyflavone) is one of the major flavonoids found in Scutellaria baicalensis (Chinese Skullcap), Scutellaria radix, and Terminalia arjuna. S. baicalensis, an important source of flavonoids, has long been used as a folk medicine for hepatitis and inflammatory diseases in China and Japan (Huang et al., 1994). Of the flavones, baicalein has gained attention as a therapeutic agent due to various biological properties by demonstrating a potent effect on inflammatory responses through the inhibition of cytokines and related genes (Sekiya and Okuda, 1982; Kubo et al., 1984; Huang et al., 1994; Chen et al., 2004). In addition, baicalein is beneficial in numerous pathological conditions such as cancer (Ikemoto et al., 2000), oxidation (Shao et al., 2002), and metabolic disorders (Pu et al., 1984; Huang et al., 1994; Chen et al., 2004). In addition, baicalein is beneficial in numerous pathological conditions such as cancer (Ikemoto et al., 2000), oxidation (Shao et al., 2002), and metabolic disorders (Pu et al., 2012). It has also been reported to exhibit anti-adipogenic effects and inhibit adipocyte differentiation by enhancing COX-2 expression (Cha et al., 2006).

Obesity is a physical condition in which excessive fat accumulates, resulting in certain health risks. This global health problem is a major risk factor for metabolic diseases such as type 2 diabetes, insulin resistance, and atherosclerosis (Spiegelman et al., 1993). Adipogenesis is a differentiating procedure of a preadipocyte to a mature adipocyte, abnormality of which is a direct cause of obesity. Adipogenesis includes complicated mechanistic events whose processes have been established via cell line studies. Adipocyte differentiation begins by actions of differentiation inducers such as insulin, IBMX, and dexamethasone, in which early adipogenic transcription factors, including members of the Krueppel-like factor (KLF) family, Krox20 (early growth response 2, Egr2), CCAAT/enhancer binding protein-β (C/EBPβ), and CCAAT/enhancer binding protein-α (C/EBPα), are expressed (Banerjee et al., 2003; Chen et al., 2005; Oishi et al., 2005; Lowe et al., 2011). Activation of early adipogenic factors induces late adipogenic factors such as CCAAT/enhancer-binding protein-α (C/EBPα) and peroxisome proliferator-activated receptor-γ (PPARγ), which play a pivotal role in the formation of mature adipocytes (Rangwala and Lazar, 2000).

Expression of C/EBPα and PPARγ is necessary to activate lipid synthetic proteins, including adipose protein 2 (aP2) and lipin1, which play major roles in fatty acid transfer and triglyceride synthesis, respectively (Attie and Scherer, 2009). Specifically, triglyceride (TG) is a main causal molecule for fat accumulation in obesity. TG synthesis is achieved by the actions of various enzymes such as LPAAT, lipin1, and DGAT, which catalyze the TG synthetic...
pathway (Lowe et al., 2011). In addition, lipid synthetic proteins such as fatty acid synthase (FASN) and sterol regulatory element binding protein (SREBP), which are involved in fatty acid and cholesterol synthesis, also contribute to lipid homeostasis as an enzyme and transcription factor, respectively. Lipid synthetic processes such as adipogenesis require cell growth and proliferation, which are accompanied by cell cycle progression. When post-confluent cells are exposed to inducers of differentiation, growth-arrested cells begin cell cycle progression by entering into S phase from G1/G0 phase, thus contributing to differentiation. The cell cycle is controlled by cyclin-dependent kinases (CDKs), which are responsible for cell cycle progression. Thus, regulation of the cell cycle can be a way to control adipocyte differentiation (Reichert and Eick, 1999). Meanwhile, m-TOR has also been shown to be one of the master regulators of cell growth and metabolism (Ma and Blenis, 2009); m-TOR positively regulates cell proliferation through the activation of anabolic processes and suppressing catabolic pathways. Recent studies suggest that m-TOR is a regulator of lipid synthesis (Laplante and Sabatini, 2009). Positive regulation of m-TOR during adipogenesis has been represented by the inhibitory effect of rapamycin, an m-TOR inhibitor, on adipocyte differentiation (Yeh et al., 1995; Bell et al., 2000; Gagnon et al., 2001).

The zebrafish (Danio rerio) has gained attention as a new strategy for in vivo studies on diseases. It is useful for in vivo observations of developmental and physiological processes since it provides optical transparency during early adulthood. Recent studies suggest that genes associated with lipid metabolism, including adipogenic factors and signaling pathways, are conserved in mammals (Farber et al., 2001; Pickart et al., 2006). The zebrafish is highly efficient in genetic and chemical screens (Patton and Zon, 2001) used to identify new genes and molecules that regulate adipocyte differentiation and energy metabolism.

In this study, we investigated the effect of baicalein on lipid accumulation by focusing on the early phase of adipogenesis, cell cycle arrest, lipogenesis, and m-TOR signaling in 3T3-L1 cells, and identified an anti-adipogenic effect of baicalein in zebrafish.

2. Materials and methods

2.1. Chemicals

Baicalein (5,6,7-trihydroxyflavone (C15H10O5)) was purchased from Alfa Aesar (Seoul, Korea) (Fig. 1A). Dulbecco’s modified Eagle’s medium (DMEM), bovine calf serum (BCS), fetal bovine serum (FBS), penicillin–streptomycin (P/S), phosphate-buffered saline (PBS), and trypsin–EDTA were purchased from Gibco (Gaithersburg, MD, USA). Dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX), insulin, Oil Red O, and all other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.2. Cytotoxicity

3T3-L1 cells (~1 × 10⁴ cells per well) were treated with baicalein in 96-well plates. 2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carbox-anilide (XTT) reagent was added to the culture after a 24–48 h incubation. The cytotoxicity of baicalein was monitored every 2 h at wavelengths of 450 nm and 690 nm, respectively.

2.3. Cell culture

3T3-L1 preadipocytes (CL-173; American Type Culture Collection, Manassas, VA, USA) were cultured, maintained, and differentiated. Briefly, cells were plated and grown in DMEM with 3.7 g/L sodium bicarbonate, 1% P/S, and 10% BCS. Adipocyte differentiation was induced by treating 2-day post-confluent cells with 10% BCS, and grown in DMEM with 3.7 g/L sodium bicarbonate, 1% P/S, and 10% BCS. Adipocyte differentiation was induced by treating 2-day post-confluent cells with 10% BCS. The quantities of treated samples were adjusted using 1% media.

2.4. Oil Red O staining

The extent of differentiation reflected by the amount of lipid accumulation was determined at day 8 by Oil Red O staining. Briefly, cells were fixed in 10% formaldehyde in PBS for 1 h, washed with distilled water, and dried completely. Cells were stained with 0.5% Oil Red O solution in 60:40 (v/v) isopropanol:H₂O for 30 min at room temperature, washed four times with water, and dried. Differentiation was monitored under a microscope and quantified by elution with isopropanol and optical density measurements at 490 nm (Lee et al., 2012).

2.5. Cell cycle analysis by fluorescence-activated cell sorting (FACS)

To investigate the effects of baicalein on cell cycle progression during 3T3-L1 adipocyte differentiation, a BD FACS Calibur™ Flow Cytometer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was used. For this analysis, preadipocytes were differentiated for 18 h and 24 h with or without baicalein. 3T3-L1 cells were fixed in 70% ethanol for at least 2 h and washed with PBS. Cells were stained with 300 µL propidium iodide (PI) solution (50 µg/mL) containing 20 µL RNase (10 mg/mL) and incubated at 37 °C for 30–45 min. After staining, the cell cycle was monitored using a flow cytometer.

2.6. RNA extraction and semiquantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from 3T3-L1 adipocytes and zebrafish (20 dpf) using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. RNA samples with OD₂₆₀/OD₂₈₀ ratios > 2.0 were used for semiquantitative RT-PCR. One microgram of total RNA was used to produce cDNA. Oligonucleotide primer sequences were as Table 1. PCR products were electrophoresed on 1.5% (v/v) agarose gels, stained with ethidium bromide, and photographed. Expression levels were quantified via scanning with ImageJ gel documentation and analysis system (NIH, Bethesda, MD, USA).

2.7. Western blot analysis

Protein extracts (30 or 50 µg) were separated via sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to a nitrocellulose membrane. Membranes were blocked and immunoblotted with primary antibodies specific for β-actin, C/EBPα, PPARγ, αP2, LPAAT1, lipin1, DGAT1, SREBP-1, FASN, cycloA, cyclinD1, cycline, m-TOR, p-m-TOR, p70S6K, and p-p70S6K.
Secondary antibodies conjugated with horseradish peroxidase (1:1000) were applied for 1 h. Bands were visualized by enhanced chemiluminescence, and proteins were detected with LAS image software (Fuji, New York, NY, USA).

2.8. Zebrafish maintenance

Zebrafish eggs were obtained from Chungnam University (Daejeon, Korea) and raised in egg water (0.6 g/L sea salt) under a 14 h light/10 h dark cycle at a constant temperature of 28 ± 0.5 °C. Fertilized embryos were plated in 100-mm-diameter Petri dishes (with 20 mL egg water) through day 5 postfertilization (dpf) at 50 embryos per dish. At 5 dpf, embryos were transferred to 200 mL egg water and fed hardboiled egg yolk as a high-fat diet (HFD) with baicalein (1.5625, 3.125, 6.25 μM) or curcumin (2.5 μM) until 20 dpf.

2.9. Nile Red staining

Nile Red (Invitrogen N-1142) stock solution was made in acetone (1.25 mg/mL) and stored in the dark at −20 °C. Zebrafish were transferred to Nile Red at a final working concentration of 0.5 μg/mL egg water and incubated for 30 min in the dark at room temperature. The zebrafish were anesthetized in Tricain (Sigma MS-222) at 200 μL/L and mounted in 3% methylcellulose (Flynn et al., 2009). An ECLIPSE E800 (Nikon, Tokyo, Japan) fluorescence microscope was used for green fluorescent imaging.

2.10. Triglyceride (TG) analysis in zebrafish

A triglyceride assay kit (Zen-Bio, Durham, NC, USA) was used to determine the effect of baicalein on TG accumulation in vivo. At 20 dpf, zebrafish were collected and washed with 150 μL wash buffer (TG-5-RB). The wash buffer was removed, and 15 μL lysis buffer was added to each tube and incubated at 37–50 °C for 20 min. Wash buffer (135 μL) and 20 μL reagent B were then added to the samples and incubated at 37 °C for 2 h (sonication was performed if needed). After the addition of 100 μL reagent A, samples were incubated at room temperature for 15 min and read at 540 nm.

2.11. Statistical analysis

All experiments were repeated three times. The results were analyzed using an analysis of variance (ANOVA) and Duncan’s multiple range test. A P-value of <0.05 was considered statistically significant (SAS Institute, NC, USA).

3. Results and discussion

3.1. Effect of baicalein on cell viability

The effect of baicalein on 3T3-L1 adipocyte cell viability was analyzed by an XTT assay. Baicalein was nontoxic to 3T3-L1 cells within the experimental range of concentrations, demonstrating over 90% cell viability compared with the control (Fig. 1B). No change in cell morphology was observed in the microscopic analysis (data not shown). Based on these data, baicalein concentrations of 3.125, 6.25, and 12.5 μM were chosen for subsequent experiments.

3.2. Baicalein inhibits lipid accumulation during adipogenesis

The effects of baicalein on lipid accumulation were evaluated during differentiation of 3T3-L1 preadipocytes for 8 days. Baicalein treatment induced lipid accumulation suppression, as shown by Oil Red O staining in a dose-dependent manner (Fig. 2A). Specifically, 12.5 μM baicalein showed 80% inhibition of lipid accumulation during differentiation. To identify the specific inhibitory stages during adipogenic processes, baicalein was used to treat various adipogenic stages. Adipogenic processes can be divided into three classes: early stage (days 0–2), at which point adipocyte differentiation was initiated by a hormonal cocktail treatment; postmitotic clonal stage (days 2–4); and terminal stage (after day 4), when the mature adipocyte is formed. Differentiating adipocyte cells exposed to the MDI were treated with 12.5 μM baicalein at various time points during adipogenesis, as illustrated in Fig. 2B. After 6 days of differentiation, cell differentiation was determined by Oil Red O staining (Fig. 2C). Our results show that lipid accumulation was greatly inhibited when baicalein was added during days 0–2, days 0–4, and days 0–6. However, treatment with baicalein after day 2 did not show significant inhibition of differentiation compared with the control (Fig. 2C). This result indicates that baicalein mainly inhibited lipid accumulation during the early stage of differentiation. Many studies have reported that the anti-adipogenic effect of natural compounds was associated with inhibition of an early adipogenic stage (Kim et al., 2011). Since differentiating processes require operations of sequential adipogenic programs, this suppression of early events during differentiation is thought to negatively affect the adipogenic processes necessary to accomplish adipocyte differentiation. Numerous genetic and cellular changes occur in early adipogenic stages. Early phases of differentiation include increased cell proliferation and expression of various adipogenic transcription factors. Therefore, we next investigated the effects of baicalein on early adipogenic factor expression during differentiation.

3.3. Baicalein inhibits mRNA expression of early adipogenic transcription factors during differentiation

Adipogenic differentiation was initiated by a hormonal cocktail (MDI) treatment, which accompanies the induction of various transcription factors. Early adipogenic transcription factors such as C/EBPβ, C/EBPδ, PPARγ, and Krox 20 begin to be expressed for differentiation promotion within a couple hours following MDI treatment (Darlington et al., 1998; Chen et al., 2005; Birsoy et al., 2008; Lowe et al., 2011). Unlike these early adipogenic factors that stimulate adipogenesis, KLF2 and Pref-1, which are anti-adipogenic factors, play negative roles in adipogenesis (Banerjee et al., 2003; Wang et al., 2006). To determine whether these early adipogenic factors...
are regulated by baicalein, we analyzed mRNA expression levels in the absence or presence of baicalein during early differentiation. Baicalein inhibited mRNA expression of pro-early factors, including C/EBPβ and C/EBPδ, KLF5, and Krox20, in a dose-dependent manner (Fig. 3A and B). However, KLF2 and Pref-1 mRNAs were increased compared with the control (Fig. 3A and B). This result shows that baicalein suppresses early adipogenesis by regulating early adipogenic factors.

Early adipogenic factors directly or indirectly affect the expression of adipogenic factors, such as PPARγ and C/EBPα, which are master genes required for adipogenesis (Rangwala and Lazar, 2000). As expected, baicalein also inhibited major adipogenic factors including PPARγ and C/EBPα, as well as their target gene, aP2, at the protein level (Fig. 4A). Our data suggest that baicalein inhibits adipogenesis by suppressing adipogenic factors through the regulation of early adipogenic factors.

3.4. Baicalein suppresses expression of lipogenesis-related factors during differentiation

Lipid storage, a defining characteristic of adipocytes, is accomplished via lipogenic processes represented by the synthesis of TGs and fatty acids during adipocyte differentiation. Numerous enzymes are associated with TG and fatty acid synthesis, and are

![Fig. 2.](image)

The baicalein inhibits lipid accumulation and adipogenesis especially early stage of differentiation. 3T3-L1 preadipocyte was differentiated during 6 days with baicalein (3.125, 6.25 and 12.5 μM) treatment every 2 days, and performed ORO staining (A). 2 day after fully confluent 3T3-L1 preadipocytes were incubated in FBS media with MDI cocktail to induce adipocyte differentiation for 2 days, in day 2–4, adipocytes were incubated in FBS media with insulin and in day 4–6, adipocytes were incubated in FBS media. Differentiated adipocytes were treated with 12.5 μM baicalein at the indicated time points, as shown in (B), followed by ORO staining (C). The accumulated lipid was evaluated by photographs and absorbance at 490 nm. These data were measured as the standard deviation of three replicates. Results were analyzed by ANOVA and Duncan’s test (p < 0.05); (CON; control, fully differentiated without treatment, ND; nondifferentiation, preadipocyte, MDI; differentiation cocktail, IBMX (isobutylmethylxanthine) + dexamethasone + insulin.)

![Fig. 3.](image)

The effect of the baicalein on the early adipogenic factor mRNA expressions. Differentiating 3T3-L1 adipocytes were cultured with baicalein (3.125, 6.25 and 12.5 μM) for 2 to 4hr. Total cellular RNA was extracted and performed RT-PCR analysis (A) and quantitative analysis of RT-PCR gene expressions (B). These data were measured as the standard deviation of three replicates.
interrelated with adipogenic transcription factors (Zhang et al., 2008). Glycerol 3-phosphate acyltransferase converts glyceralddehyde 3-phosphate (G3P), a metabolite of glucose, to lysophosphatidic acid (LPA), and LPA is turned to phosphatidic acid (PA), a biosynthetic precursor of acylglycerols, by LPAAT. Lipin1 catalyzes the conversion of PA into diacylglycerol (DAG), from which TG is synthesized by DGAT (Coleman and Mashek, 2011; Lowe et al., 2011). We determined the expression levels of these TG synthetic proteins in the presence or absence of baicalein. Our data showed that baicalein inhibited the expression of LPAAT, DGAT1, and lipin1 in a dose-dependent manner (Fig. 4B). This result demonstrates baicalein inhibits lipid accumulation by suppressing the expression of TG synthetic enzymes. Lipin 1 and adipogenic transcription factors such as PPARγ and C/EBPα are positively inter-regulated (Zhang et al., 2008; Rosalind and Douglas, 2011). Fatty acid (FA) synthesis also accompanies with actions of many factors. We evaluated expression levels of fatty acid synthase (FASN) and SREBP1 as a representative protein and transcription factor, respectively, for FA synthesis. Baicalein decreased expression levels of SREBP1 and FASN proteins (Fig. 4C), suggesting that baicalein negatively regulates FA synthesis by downregulating SREBP1 and FASN, which are major fatty acid synthetic factors. SREBP1, a master gene regulating FA and cholesterol synthesis, is positively inter-regulated with PPARγ, a key transcriptional regulator of adipocyte differentiation (Rangwala and Lazar, 2000). Our data show that baicalein inhibits lipid accumulation during adipocyte differentiation through the regulation of multiple adipogenic factors. However, whether baicalein regulates a specific factor that can affect other regulators, or whether it exerts influence on various factors at the same time, is yet to be known. Our results suggest that baicalein-induced regulation of early adipogenic factors, which can affect other adipogenic factors and enzymes, could be one of the major causes for the inhibition of lipid accumulation during adipogenesis.

3.5. Baicalein delays cell cycle progression in 3T3-L1 cells

Cell proliferation is an important factor for adipocyte differentiation. Actually, preadipocyte undergoes a great increase in cell number within 48 h after MDI treatment and is termed mitotic clonal expansion (MCE) (Lowe et al., 2011). The cell cycle is a basic process for cell growth and division. To determine whether baicalein regulates the cell cycle after adipogenic induction, the effect of baicalein on cell growth during differentiation was analyzed via FACS analysis (Jayat and Ratinaud, 1993; Tang et al., 2003). FACS data showed that baicalein (especially 12.5 μM) arrested cell cycle progression at the G0/G1 phase (Fig. 5A and B). After the induction of differentiation, growth-arrested preadipocytes initiated cell cycle progression through the transition of G1 to S phase at 18 h (Tang and Lane, 1999). Baicalein (12.5 μM) blocked the G1 to S phase transition by exhibiting similar cell population as undifferentiated preadipocytes (Fig. 5A and B). At 24 h, baicalein also delayed cell progression at each phase of the cell cycle compared to the control (Fig. 5A and B). Our histogram data were supported by cyclin analysis via immunoblotting (Fig. 5C). The expression of cyclin D and E, which are involved in the G1 to S phase, decreased in the baicalein-treated group (12.5 μM) after 18 h, and cyclin A, which is responsible for the S phase and transition of the S to G2 phase was also downregulated following baicalein treatment (12.5 μM). These results show that baicalein inhibits cyclins to suppress cell proliferation, which is required for cell differentiation. After 24 h, cyclin D expression showed a great decrease in the control group, indicating cell cycle transition with time. Our data suggest that baicalein inhibits lipid accumulation by regulating the cell cycle process. Numerous studies showed an inhibitory effect of natural compounds on adipocyte differentiation closely associated with the inhibition of cell cycle progression (Tang et al., 2003). Phytochemicals such as curcumin and sulforaphane inhibit adipogenesis through cell cycle arrest (Kim et al., 2011). Regulation of the cell cycle by baicalein is thought to negatively affect normal adipocyte differentiation such as expression of adipogenic factors.

3.6. Baicalein inhibits the m-TOR signaling pathway

m-TOR, serine/threonine kinase, plays an important role in cell proliferation and metabolism (Kim et al., 2002). m-TOR controls cell cycle progression, which is tightly associated with cell growth, via downstream pathways including S6K1 and 4E-B (Fingar et al., 2004). In addition, many studies have shown that m-TOR controls lipid biosynthesis by various mechanisms. Specifically, m-TOR has been determined to control adipogenesis in a study in which an m-TOR inhibitor, rapamycin, inhibited adipocyte differentiation and fat accumulation (Kim and Chen, 2004). We examined the effect of baicalein on the activation of m-TOR signaling. Our data showed that baicalein suppresses activation of m-TOR and a downstream effector, P6SK, by decreasing their phosphorylation (Fig. 6). This result suggests that inhibition by baicalein on adipocyte differentiation is associated with suppression of the m-TOR signaling pathway that can regulate the activity of adipogenic factors such as PPARγ.
3.7. Baicalein suppress lipid accumulation in zebrafish

To investigate the effect of baicalein on lipid accumulation in vivo, we chose zebrafish as an experimental model. Nile Red staining was performed to visualize and determine lipid accumulation in zebrafish. Zebrafish (5 dpf) were grown by high-fat diet (HFD) feeding in sea salt containing water with or without baicalein (1.5625, 3.125, 6.25 μM). Our microscopic images show that the baicalein-treated group showed significantly decreased Nile Red-derived fluorescence in a dose-dependent manner compared to the control (Fig. 7A). In particular, baicalein showed an inhibitory effect on lipid accumulation of over 30% in 6.25 μM compared to the control group. Curcumin (CCM, 2.5 μM), an anti-adipogenic phytochemical (Kim et al., 2011), inhibited lipid accumulation by about 20% (Fig. 7A). This result correlates with the in vitro data. The Zebrafish has received considerable interest as an animal model in recent years and been found to share a genetic background and physiologic responses with those of mammals in various research areas (Ho et al., 2004). In particular, its adipogenic genes are conserved in mammals, allowing a similar physiologic status (Kaderet et al., 2008; Ho et al., 2004). To determine the effects of baicalein on adipogenic genes in zebrafish, we analyzed mRNA expression of adipogenic genes including PPARγ, C/EBPα, aP2-a, aP2-b, SREBP-1 and SREBP-2. Baicalein suppressed gene expression of these adipogenic genes in a dose-dependent manner (Fig. 7B). In addition, quantitative analysis of accumulated triglycerol levels showed that baicalein inhibited triglycerol levels dose-dependently (Fig. 7C). Our results show that baicalein inhibits lipid accumulation via the suppression of adipogenic factors in zebrafish, consistent with cell line data.

Only a few studies have been reported on natural compounds demonstrating inhibitory effects on lipid accumulation in zebrafish. A recent study showed an inhibitory effect of green tea extract on adiposity and lipid accumulation in diet-induced obese zebrafish (Hasumura et al., 2012). Our study revealed an inhibitory
effect of baicalein on lipid accumulation in a zebrafish cell line, but further mechanistic studies are needed.

4. Conclusion

In this study, we investigated the inhibitory effects of baicalein, a naturally occurring compound in plants, on lipid accumulation in cells and zebrafish. We found that baicalein effectively suppressed adipogenic lipid accumulation in both models. An anti-adipogenic effect of baicalein was achieved through the inhibition of early adipogenic factors and cell cycle arrest in an early adipogenic stage. Also, our data indicate that inhibition of m-TOR signaling is associated with the anti-adipogenic properties of baicalein. Thus, this study showed how baicalein inhibits adipogenesis in a cell line as well as in zebrafish. The present study will provide useful data for the development of antiobesity agents using baicalein.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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