이화여자대학교 대학원
2015 학년도
석사학위 청구논문

Suppression of PPARγ-induced adipogenesis by AQ

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2016

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이 논문을 석사학위 논문으로 제출함

2016 년 1 월

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List of Abbreviations

aP2: Adipocyte protein 2, a fatty acid binding protein

AQ: Amodiaquine

C/EBP: CCAAT/enhancer-binding protein

CQ: Chloroquine

DAPI: 4′, 6-Diamidino-2-phenylindole

Dex: Dexamethasone

DMEM: Dulbecco′s modified Eagle′s medium

FAS: Fatty acid synthase

FBS: Fetal bovine serum

GLUT: Glucose transporter

MT: Mutant type

PGC1α: Peroxisome proliferator-activated receptor gamma co activator 1-alpha

PPAR: Peroxisome proliferator activated receptor

PPRE: Peroxisome proliferator-activated receptor-responsive element

Rosi: Rosiglitazone

SREBP: Sterol regulatory the element binding protein

TAZ: Transcriptional coactivator with PDZ-binding motif

WT: Wild type

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Abstract

Obesity is considered one of the leading causes of life-threatening diseases. There have been many trials to develop anti-obesity drugs, which still have limited efficacies and adverse side-effects. CQ has been widely used for malarial infection and is reported to have anti-adipogenic effect. In this study, we have examined the anti-adipogenic activity of AQ that is a derivative of CQ and has more potency for treating malarial infection. As CQ inhibited adipogenic differentiation, AQ dose-dependently suppressed adipogenic gene expression and oil deposition in mature adipocytes. AQ suppressed adipogenesis more potently than CQ. While CQ revealed no effect on the expression and activity of PPARγ, AQ substantially decreased protein expression of PPARγ. In addition, AQ inhibited PPARγ activity having no suppression of DNA-binding activity of PPARγ. Interestingly, AQ strongly enhanced cytosolic localization of PPARγ, resulting in suppression of PPARγ activity. CQ had no significant effect on PPARγ localization but increased cytosolic expression of PGC1α, a PPARγ coactivator. These results demonstrate that AQ suppressed PPARγ-mediated adipogenic differentiation more potently than CQ. AQ may directly suppress PPARγ function by increasing cytoplasmic translocation of PPARγ, while CQ indirectly suppresses PPARγ activity by inhibiting nuclear PGC1α expression, suggesting therapeutic potential of AQ and CQ for treating obesity.

I. Introduction

1. Obesity

Obesity is a result of an imbalance between food ingestion and energy consumption as obtained from the storage of energy as fat, primarily in adipose tissue (Bray and Tartaglia, 2000). The obesity is a staple health risk factor that contributes considerably to the development of a lot of human diseases, including depression, type 2 diabetes mellitus, hypertension, heart diseases, and cancer (Billington, 2000) (Figure 1). The occurrence rate of obesity has increased suddenly to the recent decades all over the world (Ng et al., 2014) (Figure 2). The obesity is one of the diseases caused by over-consumption of food and lack of exercise, which exerts negative effect on human life. Thus, it is one of the most serious public health problems of the 21st century. The obesity are urgently needed to effective measures to prevent (Goossens, 2008; Greenberg and Obin, 2006; Paramsothy et al., 2009).

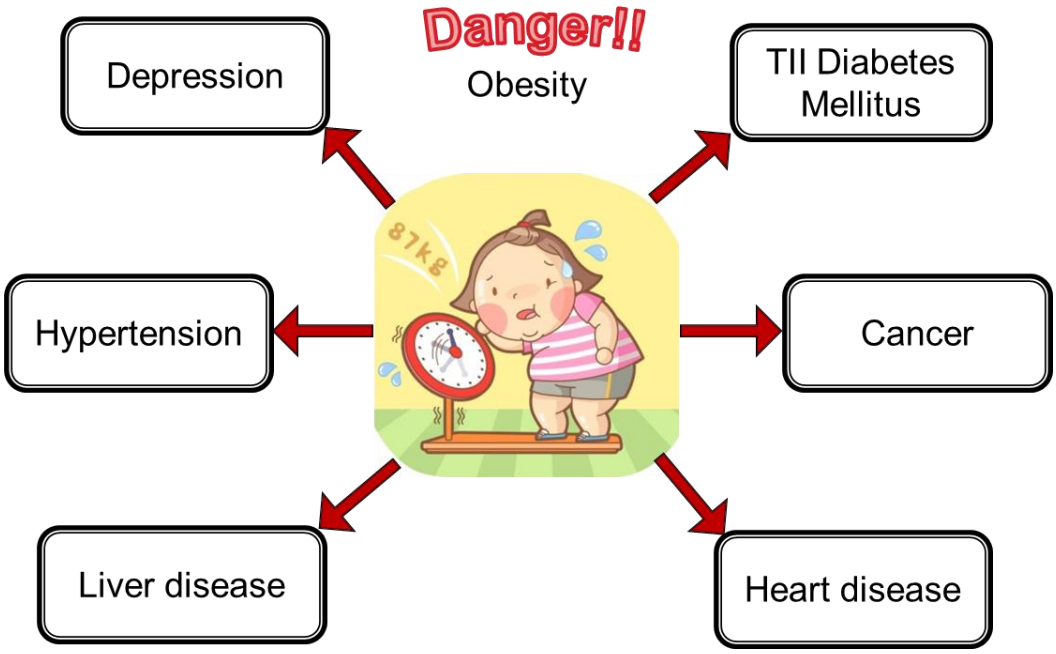


Figure 1. Development of obesity-related diseases in adults (Must et al., 1999)

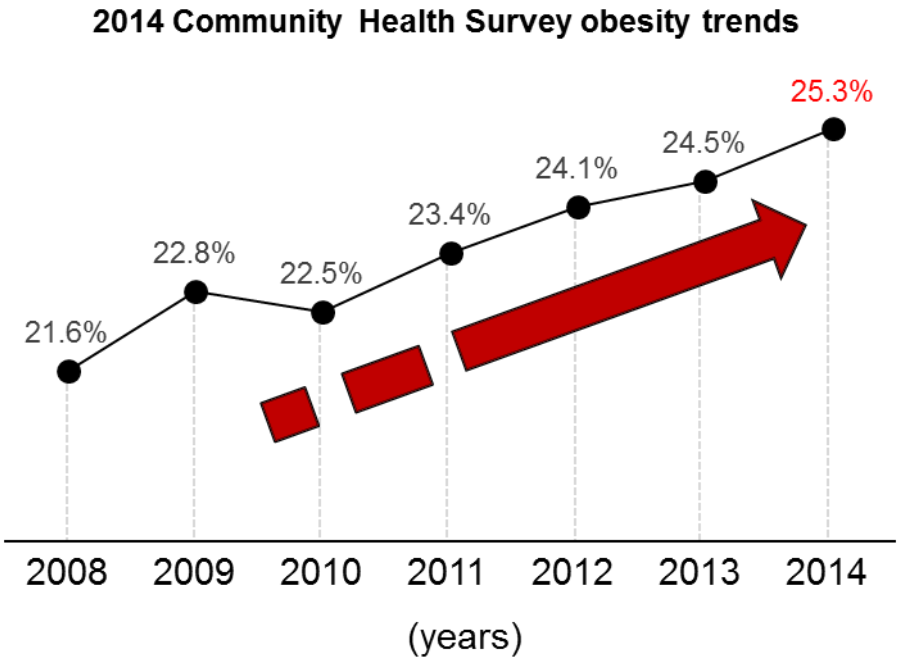


Figure 2. Increasing trends of the population of obesity in last 7 years

According to the chart, obesity rates are increasing in Korea as 21.6% in 2008 and 22.5% in 2010 (Korea Centers for Disease Control & Prevention, 2014).

2. In vitro adipogenesis model system

3T3-L1 cells have been served as well established in vitro assay system to assess lipogenesis and fat cell differentiation (Kong et al., 2009). The 3T3-L1 pre-adipocyte cell lines originally established by green and associates have greatly facilitated our knowledge of the molecular mechanisms controlling lipogenesis (Green and Kehinde, 1975). Although committed to the fat cell lineage, proliferating 3T3-L1 pre-adipocytes exert traits similar to those of other 3T3 fibroblasts. Confluent 3T3-L1 pre-adipocytes differentiate from exposure to the fat generating inducers 10% Fetal bovine serum (FBS) + Dulbecco′s modified Eagle′s medium (DMEM), dexamethasone(Dex), rosiglitazone(Rosi), and insulin. These compounds activate a lipogenesis, which occurs in two well-restricted phases. The stimulated cells rightly reenter the cell cycle and progress of at least once or twice cell-cycle divisions, a phase frequently referred to as clonal expansion (Farmer, 2006). During this step, the cells express definite adipocyte transcription factors as well as cells cycle adjusters that together assist expression of CCAAT/enhancer binding protein (C/EBP) α and Peroxisome proliferator-activated receptor (PPAR) γ (El-Jack et al., 1999). After this process, the committed cells receive terminal differentiation appeared by production of fat droplets as well as expression of several metabolic programs characteristics of mature fat cells (Figure 3).

3. Regulation of adipocyte differentiation

Mature fat cell differentiation, known as adipose formation, is a process wherein the pre-adipocytes differentiate from fat cells (Chawla et al., 1994). Lipid formation is a complex process accompanied by changes in cell morphology, hormone sensitiveness and gene expression. Lipid formation is modulated by several transcription factors, containing Sterol regulatory the element binding proteins (SREBPs), C/EBPs and PPARs (Darlington et al., 1998). After multiplication of pre-adipocytes, the differentiation is promoted by several families of transcription factors. C/EBPs, C/EBPδ and C/EBPβ are first expressed, followed by PPARγ, which in turn triggers C/EBPα. C/EBPα exerts positive feedback on PPARγ to persist differentiation (Yeh et al., 1995). SREBP1c can multiply the transcriptional activity of PPARγ (Kim and Spiegelman, 1996). Co-activator of PPARγ is Peroxisome proliferator-activated receptor gamma co activator 1-alpha (PGC1α) increases activity, while TAZ is repressing activity (Lowell, 1998, Jang et al., 2012). These factors lead the expression of genes that feature differentiated adipose cell phenotype (Figure 3). It is estimated that the structural and functional morphology formation associated with fat cell differentiation involves changes in the expression levels of ~300 proteins (Morrison and Farmer, 2000). Many of these conversions happen to the level of gene expression of a series of molecular events including several transcription factor families that exhibit diverse modes of upsweep and function.

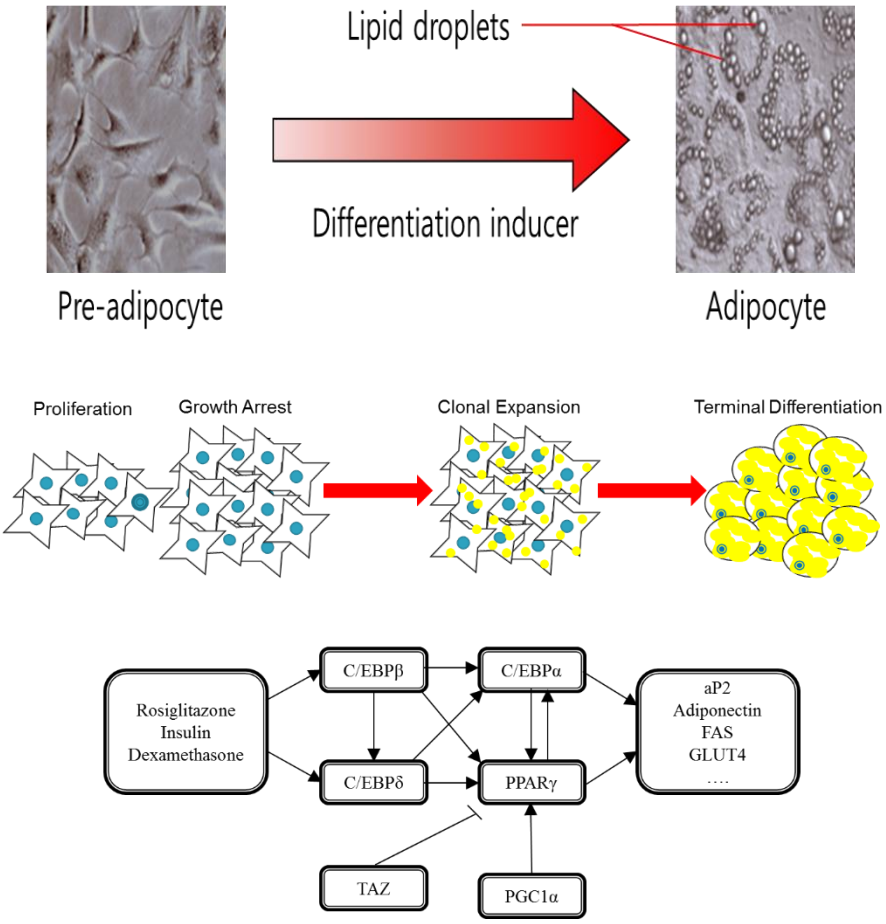


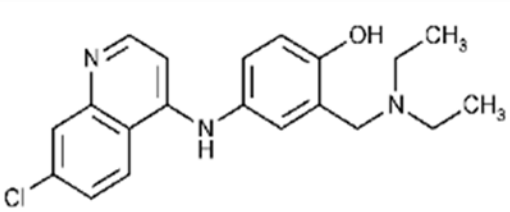
Figure3. Regulation of adipocyte differentiation by transcription factors

The process of adipocyte differentiation is divided confluence, clonal expansion, and terminal differentiation. First, the phase of confluence is conducted the phase of clonal expansion, when we handled differentiation-inducing system. At this time, it gets up the activation for giving impetus to C/EBPα and PPARγ to develop from C/EBPβ and C/EBPδ. In the process of terminal differentiation, pre-adipocyte cell was mature fat cell, if has been activated aP2, FAS, adiponectin (Mandrup and Lane, 1997).

4. Amodiaquine (AQ) and Chloroquine (CQ)

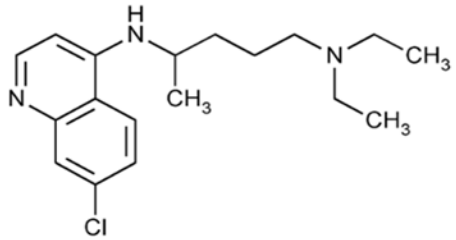
AQ, 4-[(7-chloroquinolin-4-yl) amino]-2-[(diethylamino) methyl] phenol, a 4-aminoquinoline derives that has been extensively used for process of malaria drug over the past 50 years. It is quintessential more active than the other 4-aminoquinoline, CQ, against malaria Plasmodium falciparum parasites, which are appropriately CQ tolerant. AQ is at least as effective as CQ, and is effective against some CQ-resistant strains, although resistance to AQ has been reported (Laurent et al., 1993) (Figure 4A.). CQ, N'-(7-chloroquinolin-4-yl)-N, N-diethyl-pentane-1, 4-diamine, a 4-aminoquinoline compound, is an extensively used anti-inflammatory and anti-malarial agent (Freedman, 1956; Mackenzie, 1983; Rynes, 1997). The mechanism of activation underlying the therapeutic potency of CQ is not fully known, but some biologic potency of CQ may involve the repression of lysosomal functions (Hamano et al., 2008). Albeit CQ is comparatively safe drug with fine efficacy under clinical conditions, certain ill effects increase potential concerns. Therefore, the toxicity of CQ has been laying the door of its anti-manic effects on lysosomal activity (Figure 4B). AQ and CQ has been shown some efficacy for anti-inflammatory and anti-malaria (Miller et al., 2013). And some of this information shows that CQ has anti-obesity effect (Peters et al., 2006).

(A)



Amodiaquine
(AQ)

(B)



Chloroquine
(CQ)

Figure 4. Amodiaquine (AQ) and Chloroquine (CQ)

(A) AQ, a 4-aminoquino compound related to CQ, (B) CQ, a lysosomotropic agent.

II. Materials and Methods

1. Cell culture

Pre-adipocyte 3T3-L1 (ATCC, Manassas, VA, CL-173), and human embryonic kidney (HEK) 293T cells (ATCC, CL-1573) were purchased from the American Type Culture Collection and cultured in DMEM (Invitrogen, Carlsbad, CA).

2. Adipocyte differentiation

3T3-L1 pre-adipocytes were maintained in DMEM including 10% calf serum. For adipogenic cell differentiation, the cells were blossom into confluent (day 0) monolayers and induced to differentiate from adipocytes by replacement of DMEM supplemented with 10% FBS (HyClone, Logan, UT), Dex (1 μM), insulin (5 μg/ml), and Rosi (10 μM). After, the cells were refreshed 10% FBS + DMEM including insulin (5 μg/ml) from 2 days to 4 days, and refreshed 10% FBS + DMEM on the 6 days. All of reagents for adipocyte differentiation were purchased from Sigma-Aldrich (St. Louis, MO).

3. Materials; AQ and CQ

AQ (MW = 465, Sigma-Aldrich, St, Louis, MO) and CQ (MW = 515.87 Sigma-Aldrich, St. Louis, MO) were purchased from Sigma-Aldrich. AQ was dissolved in DMSO. CQ was dissolved in PBS. To analyze the effect of AQ and CQ, 3T3-L1 cells were incubated with differentiation media including 0, 0.1, 0.5, 1, 5, 10 μM of AQ and CQ respectively. The cells were changed refreshed differentiation media every 2 days. After 7 days of differentiation, the cells were washed with PBS and analyzed for adipocyte differentiation.

4. Reporter Gene Assay

HEK 293T cells were plated at a density of 5 x 10^5 in 6-well plates and were transfected with a reporter gene (aP2-luc, PPRE-luc) and expression vector (PPARγ, PGC1α, C/EBPα, TAZ) as well as pCMV-β that expresses β-galactosidase (Invitrogen, Carlsbad, CA). Reporter gene activity was assayed using a luciferase assay kit (Promega, Madison, WI) and galactosidase assay kit, Galacto-LightTM (TROPIX, Bedford, MA). At least three independent experiments were conducted.

5. DNA pull-down assay

HEK 293T cells were transiently transfected with expression vectors for PPARγ, TAZ, C/EBPα and PGC1α and then lysed with HKMG buffer (composition: 10 mM HEPES, pH 7.9, 100 mM KCl, 5 mM MgCl2, 10% glycerol, 0.1% NP-40, and 1 mM DTT). Whole cell extracts were incubated with biotinylated double stranded DNA for 1 h, followed by incubation with HKMG buffer and resolved by SDS-PAGE followed by immunoblot assay (Hwang et al., 2005). Double-stranded DNA sequences for PPRE was 5′-caaaactaggtcaag-3′ respectively.

6. Oil Red O staining

Differentiate 3T3-L1 cells were washed with PBS and fixed with 10% formalin solution (Sigma-Aldrich, St. Louis, USA) for 1 h. The fixed cells were then washed ddH2O. And then, cells were stained with the 0.3% Oil Red O solution to RT for 10 min. Oil Red O solution was removed and immediately washed from ddH2O. And the images were acquired under the scanner. To quantity the intensity of staining, we elute Oil Red O dye by adding 1 ml of 100% isopropanol and incubate for 10 min with gently shaking. And then, the mixture was transferred to a 1.5 ml Eppendorf tube. OD was measured at 500 nm using 100% isopropanol as the blank.

7. Western blot

Whole cell extracts were harvested with ice cold lysis buffers (10% glycerol, 50 mM β-glycerophosphate, 25 mM NaF, 20 mM EGTA, 15 mM $MgCl_2$, 1 mM PMSF, 1 μg/ml protease inhibitors, 1 mM Na_3VO_4) for 15 min and centrifuged (4℃, 12000 rpm, 10 min) to gather lysate as supernatant. Whole cell extracts (30 μg) were electrophoresed on SDS-PAGE using under reducing condition. Separated proteins were transferred to nitrocellulose membranes and blocked with 3 % of BSA in TBST. NC Membrane was incubated with PPARγ. C/EBPα, β-actin (Santa Cruz Biotechnology, Santa Cruz, CA), YAP/TAZ (Cell Signaling, Beverly, MA), adiponectin (Cell Signaling, Boston, MA), PPARγ (Santa Cruz Biotechnology), PGC1α (Novus, Littleton, CO). The membrane was then washed and incubated with peroxidase-conjugated secondary the antibody for 1 h at RT. The washes were repeated, and the membrane was developed with an enhanced chemoluminescence (ECL) detection reagent (GE Healthcare Biosciences Co., Germany) according to manufacturer′s instructions.

8. Real time PCR

3T3-L1 cells were plated at the density of 1.5 x 10^5 cells/wells in 6-well culture plates. The cells were treated adipogenic media with DMSO, AQ and CQ for 7 days. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA). And then cDNA was synthesized by M-MLV reverse transcriptase (Promega, Madison, WI) using an oligodT primer. Quantitative real time-PCR was performed using an SYBR Green PCR Master Mix (Toyobo, Osaka, Japan) with an ABI Prism 7300 sequence detection system (Applied Biosystems, Foster City, CA). Primer sequences are as follows: Adiponectin, 5′-gatggcagagatggcactcc-3′, 5′-cttgccagtgctgccgtcat-3′;aP2, 5′-gtgggaacctggaagcttgtc-3′, 5′-cttcaccttcctgtcgtctgc-3′; FAS, 5′-cccttgatgaagagggatca-3′, 5′-actccacaggtgggaacaag-3′; leptin, 5′-tctccgagacctcctccatc-3′, 5′-ttccaggacgccatccag-3′;PPARγ, 5′-tgaggagaagtcacactctg-3′, 5′-tatgggtgaaactctgggag-3′;C/EBPα, 5′-aaagccaacaacgcaacgtggagac-3′, 5′-tgtccagttcacggctcag-3′;C/EBPδ, 5′-tgcgcaagagccgcgacaagg-3′, 5′-tgctccacgcgctgatgcag-3′; and C/EBPβ, 5′-accctgcggaacttgttc-3′, 5′-agggcacgcacggtggtc-3′.

9. Cytotoxicity assay

Cytotoxicity was assayed to using a cytotoxicity assay kit (EZ-Cytox, Daeil Lab Service Co., Seoul, Korea). 3T3-L1 cells were seeded at a density of 4 x 10^4 cells /wells in a 24-well plate. After 2 days, the cells were treated with different concentrations of AQ and CQ (1, 5, 10 μM) and further incubated for 1 day and 7 days, followed by cytotoxicity assay based on the kit manufacture′s protocol. The absorbance was read at 450 nm using an Elisa plate reader (Molecular Devices, Sunnyvale, CA).

10. Immunofluorescence

3T3-L1 cells were placed on poly-L-lysine-coated slides, fixed for 10 min at RT in 4% para-formaldehyde in PBS (pH 7.4). And then blocked with 5% horse serum, 0.01% sodium azide and 0.1% saponin in PBS for 30 min at RT. And incubated with a PPARγ (Affinity BioReagents, Golden, CO), TAZ (BD PharMingen, San Diego, CA), and PGC1α antibodies for 1 h at RT. Cells were washed for 1 min with PBS three times and incubated with secondary antibodies for 30 min at room temperature. And then washed for 1min with PBS three times and mounted using fluorescent mounting medium (DAKO Corporation, Carpinteria, USA) with DAPI (Roche, Indianapolis, IN) to visualize nuclei. Confocal fluorescence image was captured by using confocal microscopy (A1C Nikon, Japan).

III. Results

1. Cytotoxicity of AQ and CQ

To confirm the available treated concentration of AQ and CQ in 3T3-L1 cells, 3T3-L1 cells were incubated with AQ and CQ (0, 1, 5 and 10 μM) for day1 and 7 days. Cell toxicity was quantified using Cell cytotoxicity assay kit (EZ-CYTOX). As a result, AQ and CQ have no cytotoxicity under 10 μM in 3T3-L1 cells (Figure 5.).

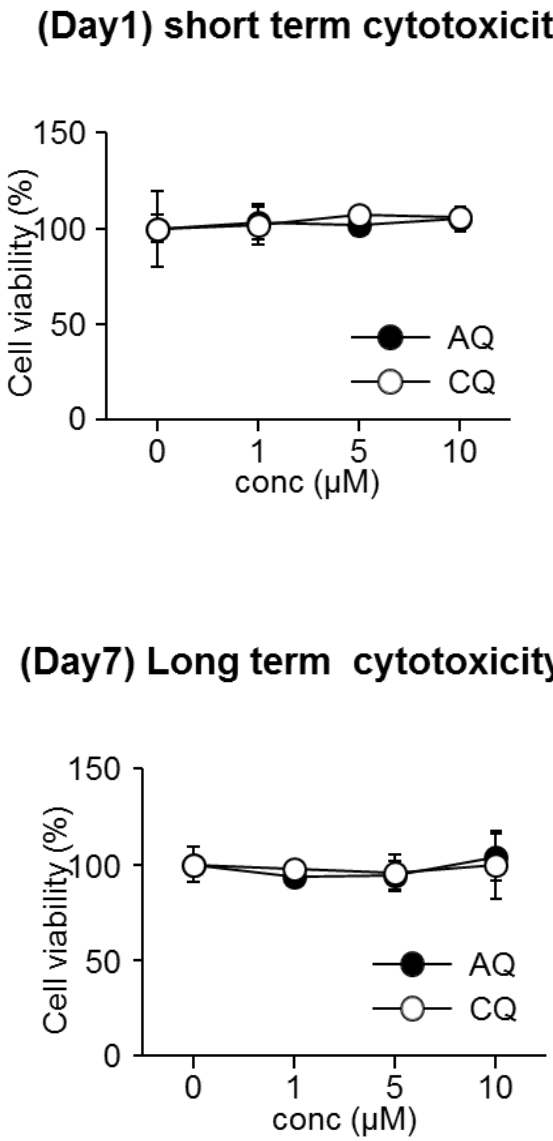


Figure 5. Cytotoxicity of AQ and CQ

Cell toxicity was determined by cytotoxicity assay. 3T3-L1 adipocytes were incubated with AQ and CQ at various concentrations for short term (1 day) and long term (7 days).

2. Suppression of adipogenic differentiation by AQ and CQ

To inquiry the inhibitory effect of AQ and CQ, we treated it at 3T3-L1 adipocyte, throughout adipocyte differentiation a span of every 2 days. 3T3-L1 pre-adipocytes differentiated with Rosi (2 μM), Dex (1 μM), insulin (5 μg/ml) and 10% FBS + DMEM. During this time, to investigate its effect on 3T3-L1 adipocyte differentiation, AQ and CQ were added to the medium at 0 day and adipocytes were stained by Oil red O staining at 7 days. Numerous intracellular lipid droplets were observed in control group. In whereas, 1, 5 and 10 μM showed significantly reduction of adipocyte differentiation. These results of AQ and CQ would diminished adipocyte differentiation (Figure 6.). In addition, treatment with AQ and CQ repressed the change of adipogenic morphology via the inhibition of intracellular lipid droplets during adipogenesis.

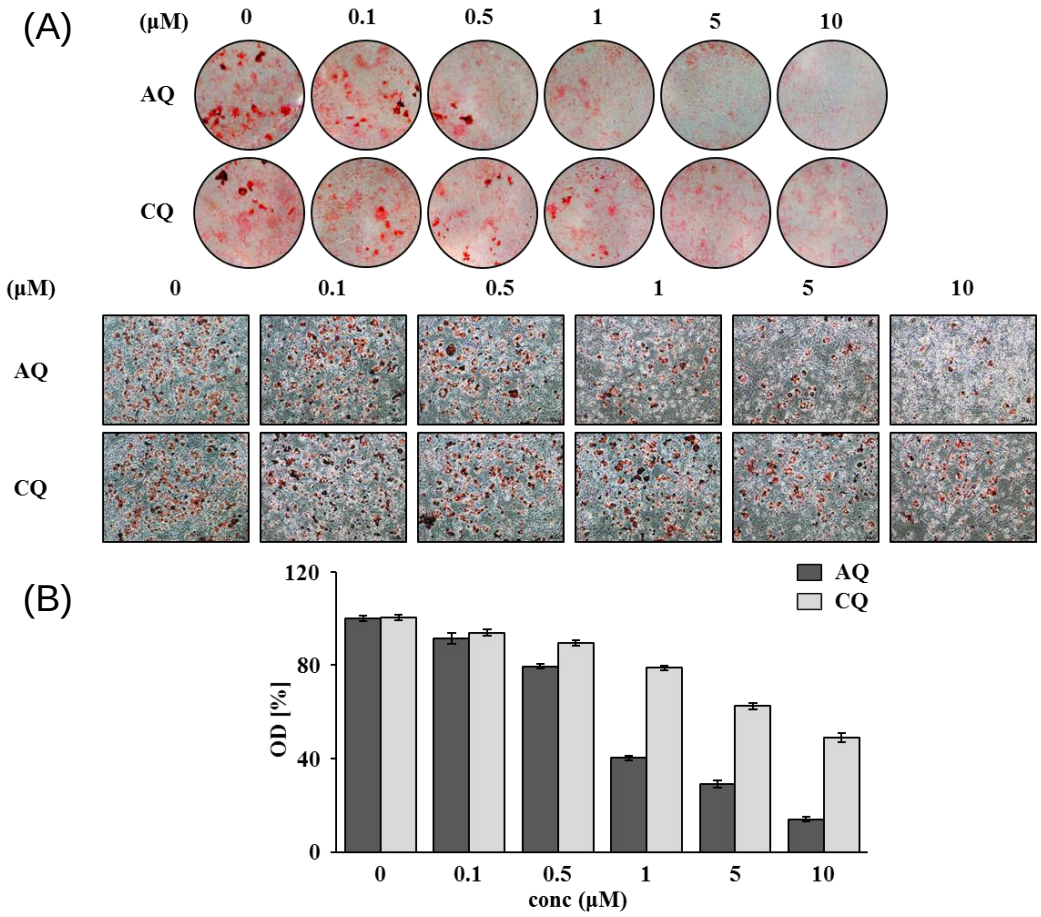


Figure 6. Suppression of adipogenic differentiation by AQ and CQ

Induction media was treated with various doses of AQ and CQ (0, 1, 5 and 10 μM) (A) Cells were stained with Oil red O. (B) Stained Oil red O lipid droplets were dissolved in 100% isopropanol and the absorbance was measured by ELSA at 500 nm.

3. Inhibition of adipogenic marker expression by AQ and CQ

Immunoblot analysis was conducted to investigate whether AQ and CQ affected the expression of adipogenic transcription factors. Treatment for AQ and CQ suppressed the expression of adiponectin compared to control. Adiponectin was reduced over 80% compared to control by AQ (1 μM) (Figure 7.). AQ significantly inhibited adiponectin expression in dose-dependent manner (0, 0.1, 0.5 and 1 μM).

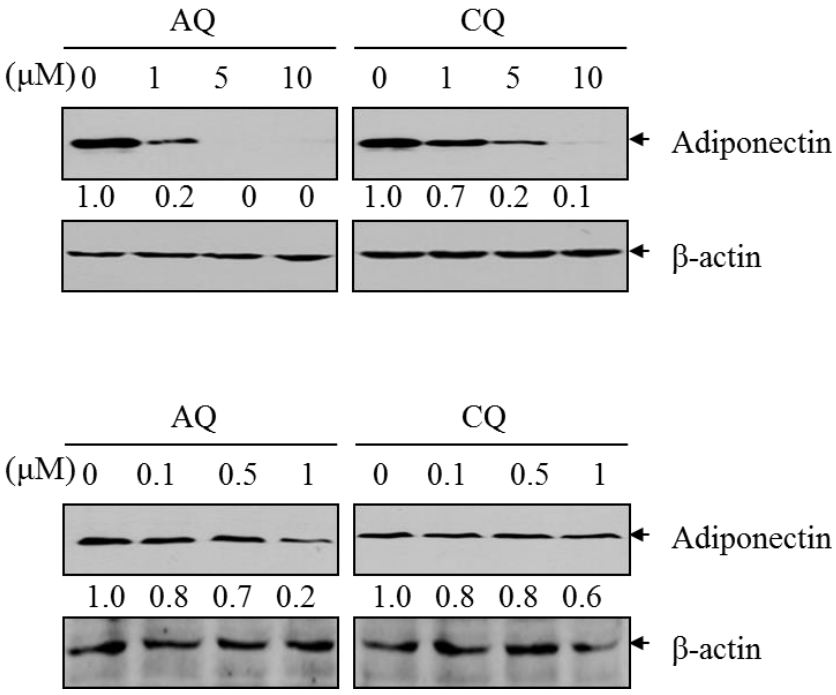


Figure 7. Inhibition of adipogenic marker expression by AQ and CQ

3T3-L1 cells were treated with AQ and CQ during adipocyte differentiation. After 7 days of differentiation Adiponectin in 3T3-L1 was analyzed by immunoblotting.

4. Inhibition of adipogenic genes by AQ and CQ

Next, we examined the expression levels of adipocyte-specific genes such as adiponectin, FAS, aP2, visfatin, resistin and leptin. After treatment with AQ and CQ in 3T3-L1 cells, mRNA was extracted on 7 days. Then, the expression of adipocyte specific genes (Figure 8.) and resistin and visfatin (Figure 9.) were investigated by RT-PCR. Adiponectin, FAS, aP2, leptin, Resistin and visfatin expression were significantly increased in Dex, insulin and Rosi stimulated differentiation. However, treatment with insulin, Dex and Rosi in the presence of AQ and CQ significantly suppressed the expression levels of adiponectin, FAS, aP2, visfatin, resistin and leptin in a dose- dependent manner.

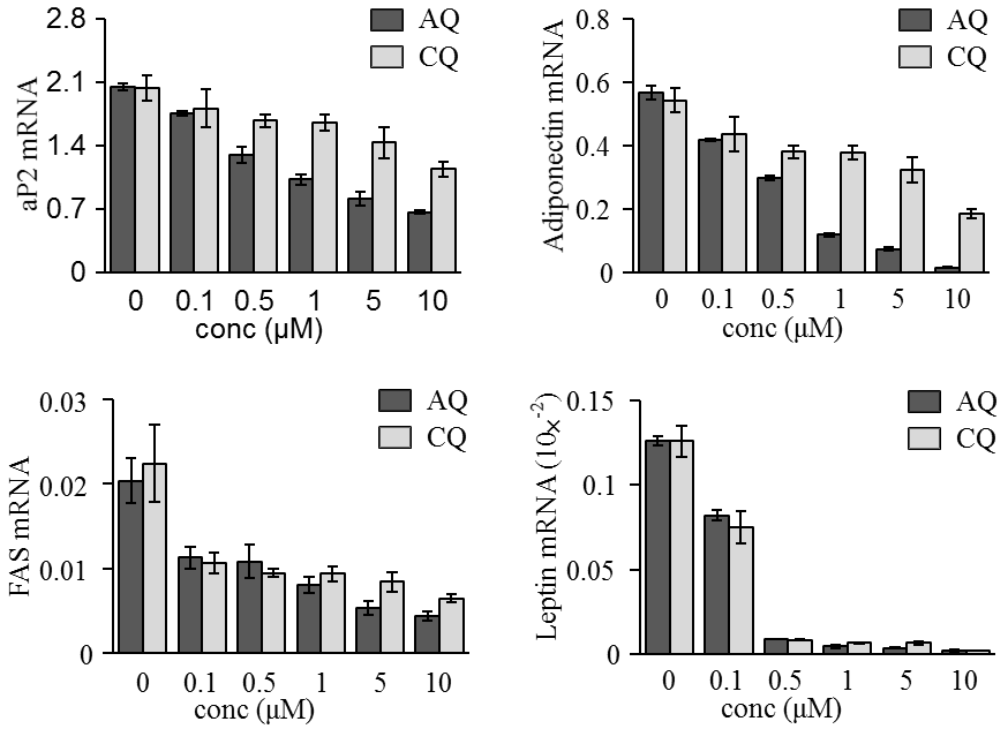
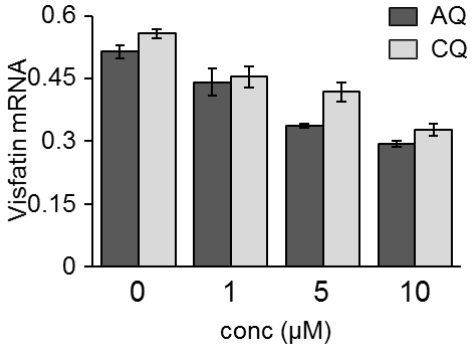


Figure 8. Inhibition of adipogenic gene transcription by AQ and CQ

3T3-L1 cells were treated with AQ and CQ during adipocyte differentiation. Total RNA was extracted from measurable real time PCR analysis. Comparative expression levels of aP2, adiponectin, Leptin, FAS transcript were determined after normalizing to β-actin transcript level.

(A)



(B)

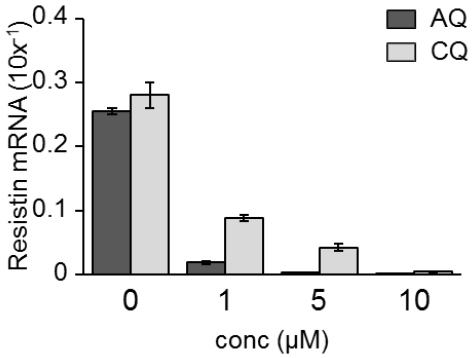


Figure 9. Decrease of adipocytokine by AQ and CQ

(A-B) 3T3-L1 cells were differentiated into mature adipocytes in the presence of AQ and CQ (0, 1, 5, 10μM) for 7 days. Total RNA was harvested from AQ and CQ treated cells and control cells and subjected to reverse transcription and real time PCR analysis. The relative expression levels of visfatin and resistin were determined after normalization to the β–actin level.

5. Suppression of PPARγ expression by AQ and CQ

Anti-adipogenic mechanisms of AQ was analyzed. CQ did not affect expression of PPARγ development of PPARγ. On the other hand, AQ decreased expression of PPARγ. But, TAZ for regulating the PPARγ activity, C/EBPα, and PGC1α is unchanged. In this experiment AQ reduced the protein level of PPARγ (Figure10).

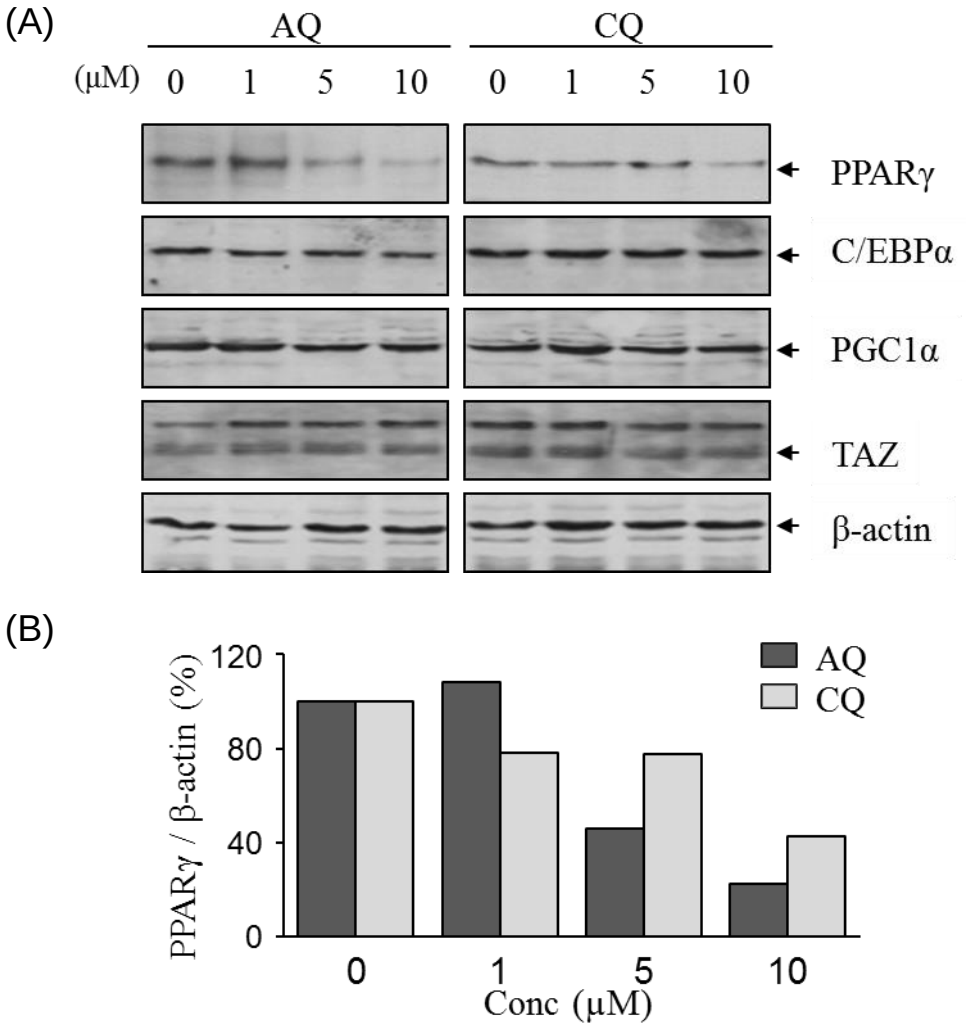


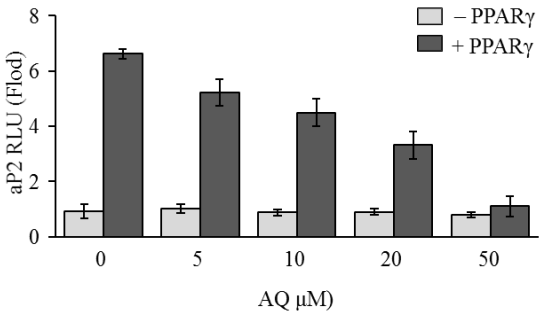
Figure 10. Suppression of PPARγ expression by AQ and CQ

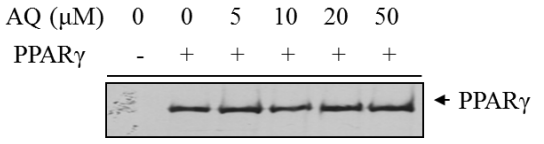
3T3-L1 cells were treated with AQ and CQ respectively. (A) The cell lysates were prepared and PPARγ, C/EBPα, PGC1α, and TAZ were analyzed by immunoblot. (B) PPARγ protein band amount was quantitatively examined by densitometry.

6. Inhibition of PPARγ -induced aP2 promoter activity by AQ

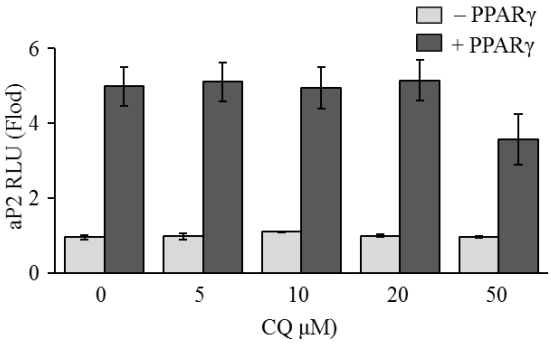
Whether AQ and CQ have an effect on PPARγ activity, some experiments are investigated. HEK 293T cell lines were transfected with aP2-luciferase reporter gene, with or without an expression vector for PPARγ. 24 h after transfection, HEK 293T cells were treated with AQ and CQ. aP2 promoter activity was enhanced by PPARγ expression. And AQ significantly repressed aP2 promoter activity. But CQ has no effect on aP2 promoter activity. In the presence of PPARγ expression AQ aP2 promoter activity in a dose dependent-manner (Figure 11.).

(A)





(B)



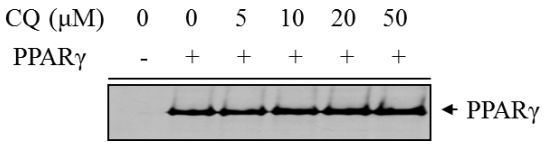


Figure 11. Inhibition of PPARγ-induced aP2 promoter activity by AQ

HEK 293T cells were transfection with the aP2-luciferase reporter construct and PPARγ expression vector, and treated with AQ and CQ for 24 h. The cells were harvested and lysed for reportergene assay.

7. No effect of AQ on DNA binding activity of PPARγ

AQ reduced protein level and activity of PPARγ. In order to uncover the inhibitory mechanisms of AQ in adipogenesis, DNA pull down assay was conducted. AQ has no effect on DNA binding of PPARγ. (Figure 12.).

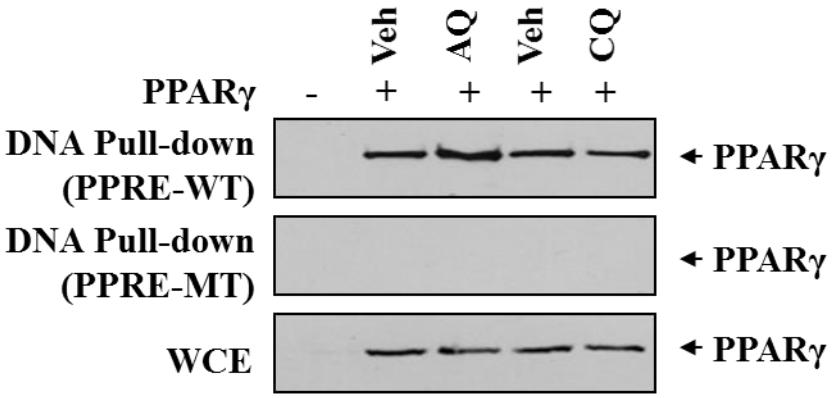


Figure 12. No effect of AQ on DNA binding activity of PPARγ

HEK 293T cells were transfected with PPARγ expression vector and after treated with AQ and CQ (50 μM) for 24 h. Whole cell extracts were incubated with biotinylated PPRE sequence and exposed to precipitation with streptavidin agarose beads. Bound proteins were eluted by boiling in SDS-loading buffer, resolved on SDS-PAGE and detected with PPARγ antibody

8. Enhanced cytoplasmic translocation of PPARγ by AQ

AQ was activity repression of PPARγ, and wasn′t effect to DNA binding of PPARγ. Other method, i.e., a nuclear translocation of PPARγ by AQ was confirmed AQ shunted nucleus expression of PPARγ as much as 71% and CQ shunted 38% on cytoplasmic. It was reduced the expression of PPARγ. Also, it was anti-adipogenic to reduced activity of PPARγ (Figure 13.).

9. Increased cytoplasmic translocation of PGC1α by AQ and CQ

PGC1α is co-activator of PPARγ would also result in a significant increase in the activity of PPARγ. By AQ, the nucleus expression of PGC1α shunted as much as 36% and CQ shunted 64% on cytoplasmic. AQ was able to shunt the nucleus expression of PPARγ and PGC1α on cytoplasmic. AQ shows anti-adipogenic more powerful effect then CQ. CQ hadn′t a dramatic effect. However, CQ was moved PGC1α nuclear expression by the cytoplasmic. CQ wasn′t change activity of PPARγ. The anti-adipogenesis occur less. (Figure 14.).

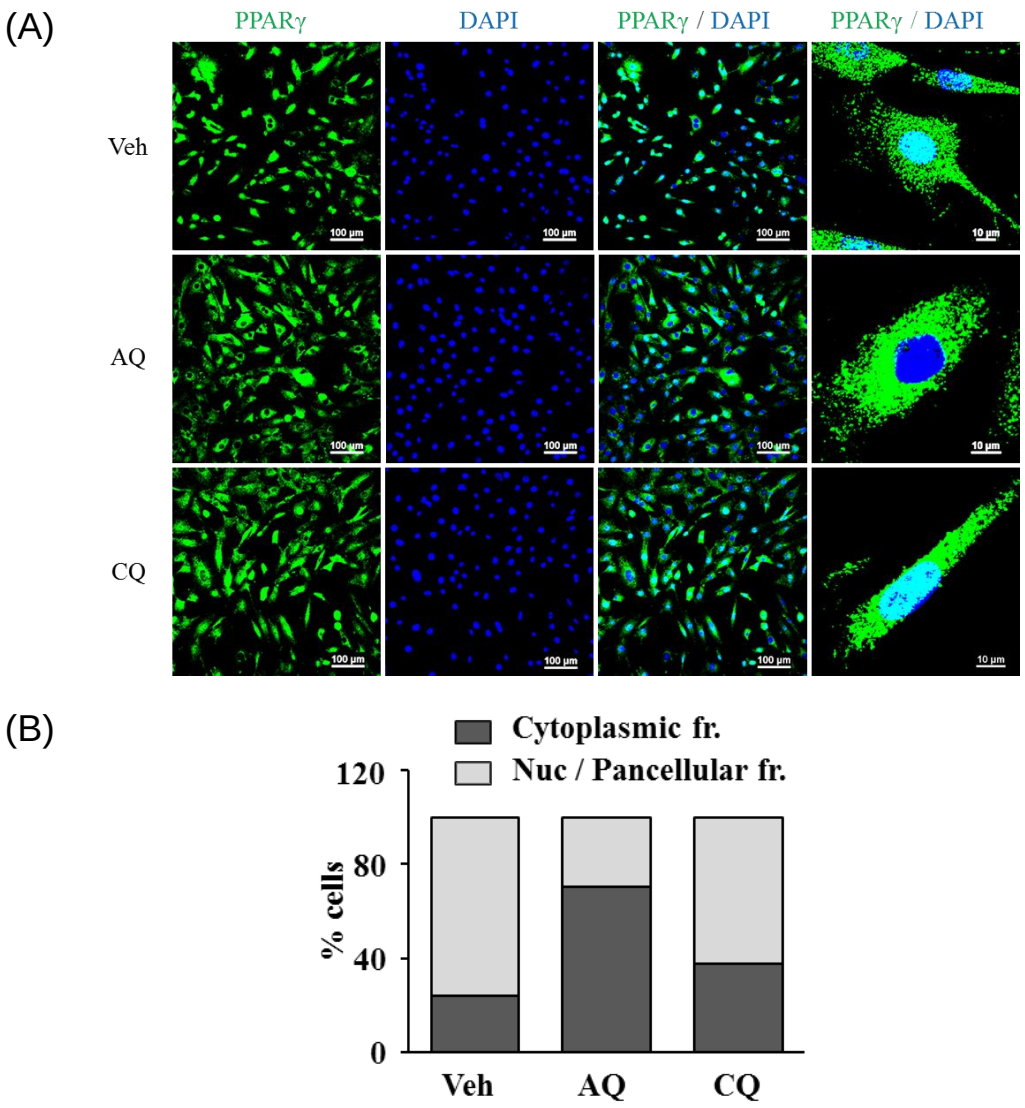


Figure 13. Enhanced cytoplasmic translocation of PPARγ by AQ

(a) Immunofluorescence images of PPARγ localization in 3T3- L1 cells. (b) Quantification of PPARγ localization in 3T3-L1 cells.

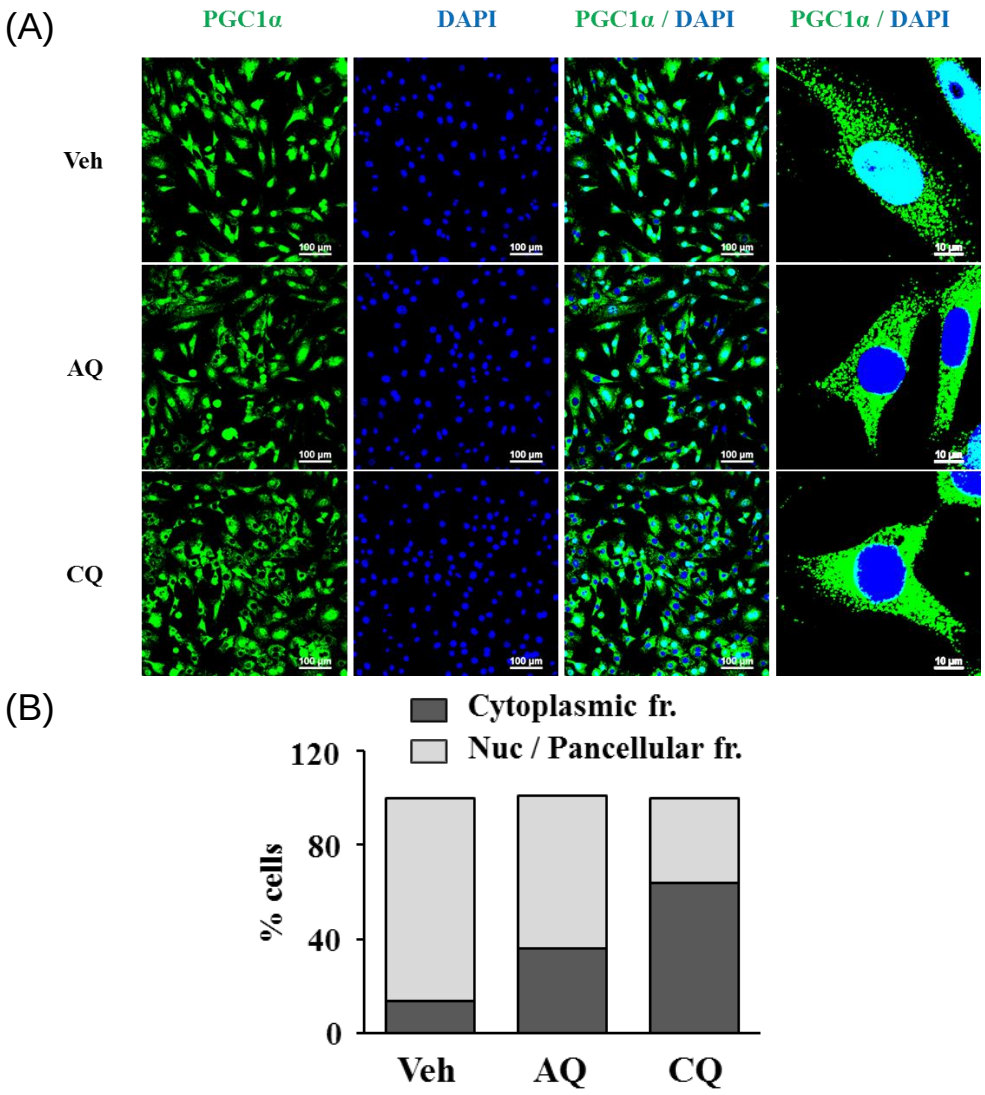


Figure 14. Increased cytoplasmic translocation of PGC1α by AQ and CQ

(a) Immunofluorescence images of PGC1α localization in 3T3- L1 cells (b) Quantification of PGC1α localization in 3T3-L1 cells.

10. No effect of AQ and CQ on TAZ localization

TAZ is the co-regulator of PPARγ suppressing PPARγ activity. We examined whether AQ and CQ have effect on TAZ localization. TAZ expression was not changed in nuclear and cytosol. (Figure 15.).

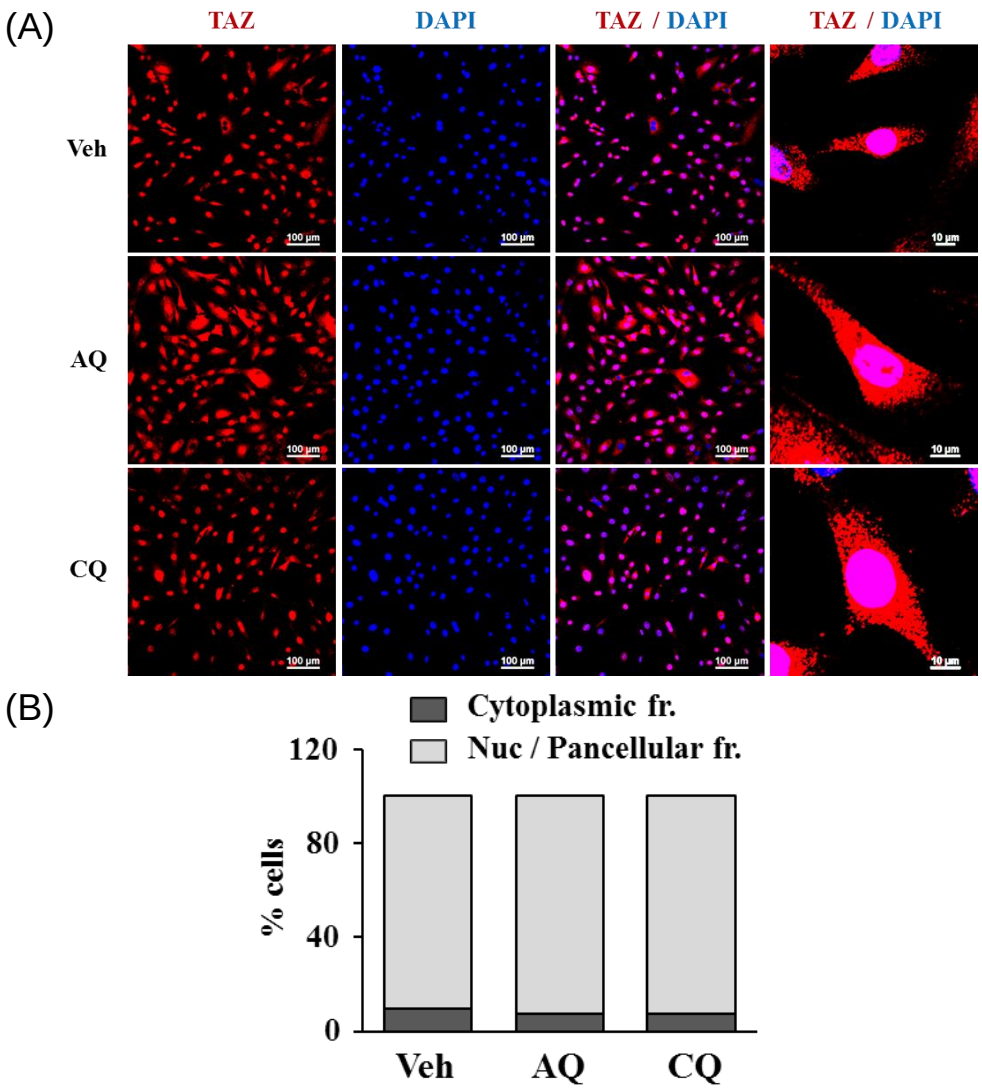


Figure 15. No effect of AQ and CQ on TAZ localization

(a) Immunofluorescence images of TAZ localization in 3T3- L1 cells (b) Quantification of TAZ localization in 3T3-L1 cells

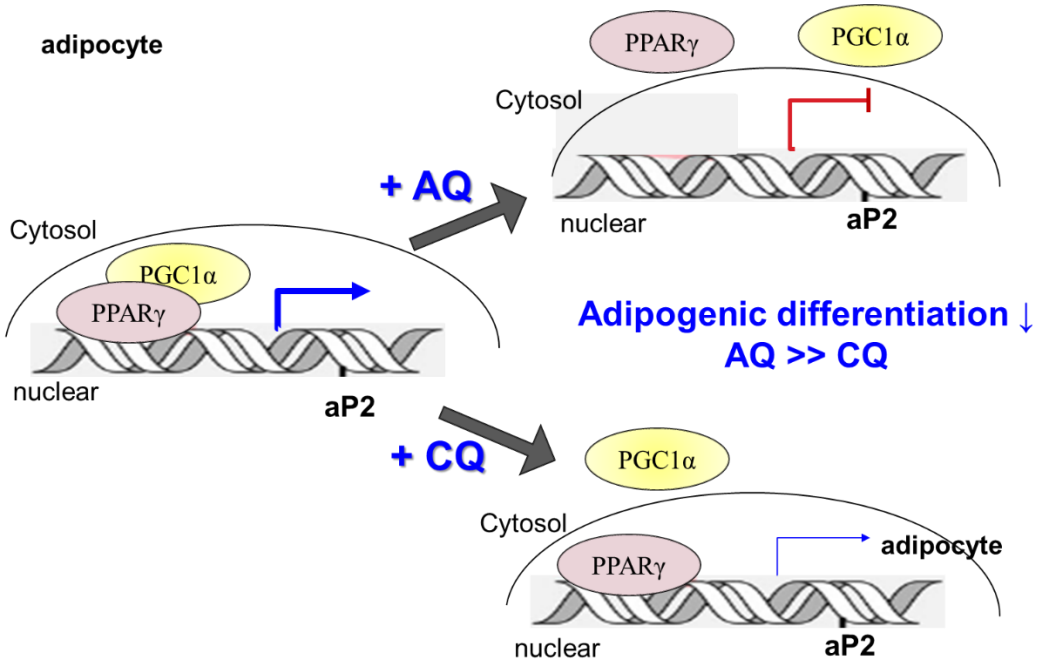


Figure 16. Model mechanism

A working model for PPARγ-mediated regulation of adipogenic differentiation. AQ strongly enhanced cytosolic localization of PPARγ and PGC1α. CQ increased cytosolic expression of PGC1α.

Discussion

The development of diet pills becomes more active by accumulation of the many studies to control adipocyte differentiation. Recently, there is a contents that CQ is anti-obesity in the paper. We progress AQ have to share mechanism of action and resistant, etc. that is a very similar CQ in this study. We have confirmed that CQ is already anti-obesity and AQ was differentiation inhibitory effect of fat cell. Before we examine its functionality. AQ is already CQ anti-obesity have confirmed that the differentiation inhibitory effect of fat cell. Before we examine its. First CQ and AQ have been confirmed by the Oil red O colors to reduce the lipid accumulation in adipocyte differentiation. In addition, adiponectin in the protein level is a terminal marker of adipocyte differentiation was a decrease in does dependent by AQ and CQ. We checked that is decreased adiponectin, FAS, aP2 and leptin in mRNA, and CQ was anti-adipogenic by the fact. We conducted the study to find out what AQ is anti-adipogenic as mechanism. The result to checked protein level of PPARγ and C/EBPα of early differentiation marker, we was confirmed to reduce protein level of PPARγ, whether C/EBPα did not change. In mRNA level, the result of AQ is not changes. Therefore, we proceed experiment to activity of PPARγ was to find out what is any. Then, we confirmed that decreased activity of PPARγ by AQ. Because AQ decreased the activity of the PPARγ, we was checked DNA binding of PPARγ what influence. So, PGC1α that is one of co-regulator of PPARγ is an increases the activity as a co-activator of PPARγ. Another one is the TAZ. TAZ reduces the activity to co-regulator of PPARγ. A protein level of PPARγ are also reduced. In addition, activity is also reduced. But it did not affect DNA binding. Thus, AQ this seems to affect the co-regulator of PPARγ now the experiment. PPARγ that is in nuclear moved all in cytosol by AQ. And PGC1α was expressed by CQ was able to confirm that the move both as cytosol. On the other hand, TAZ was not moved by AQ and CQ. In conclusion, AQ decreased the protein level of PPARγ the cytosol when expressed as a move by the pre-adipocyte differentiation expression in the nucleus of the PPARγ, also confirmed the inhibition of the activity PPARγ in this paper (Figure 16.). AQ causes a differentiation inhibitory in the adipocyte was identified as material having the potential to be developed an anti-obesity agents.

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국문초록

김태희

약학과

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비만은 대사 장애 질병의 하나로 체내에 지방이 과잉 축적된 상태를 의미하여 우울증, 고혈압, 당뇨병, 암 등의 2 차적 합병증을 유발하는 주요 원인이다. 따라서, 부작용이 적은 비만 치료제 개발이 필요로 된다. 최근 말라리아 치료제로써 사용되는 클로로퀸 (CQ)이 오토파지(Autophagy)를 조절하고 지방세포분화를 억제하는 효과가 있다고 보고되었다. 본 연구에서는 말라리아 치료제 CQ 와 그 유도체인 아모디아퀸 (AQ)를 지방세포분화에 대한 영향을 분석하고 기전을 설명하고자 하였다. AQ 와 CQ 는 3T3-L1 세포에서 독성이 보이지 않은 0, 1, 5, 10 μM 의 농도에서 지방세포분화를 확연히 억제하고 지방세포분화 마커 단백질의 발현을 억제하였으며 특히 AQ 가 CQ 보다 더 강력한 지방분화 억제 효과를 보였다. AQ 에 의한 지방세포분화 억제작용은 AQ 가 직접 PPARγ 의 발현과 억제를 억제함에 근거하며, 특히 AQ 는 세포핵 내에서의 PPARγ DNA 결합 활성에는 영향을 주지 않고 PPARγ 를 세포핵에서 세포질로의 이동을 촉진하였다. 반면, CQ 의 지방세포분화 억제는 PPARγ 발현을 일부 감소시키고 동시에 PPARγ 활성화 촉진인자인 PGC1α 의 세포질로의 이동을 촉진함으로 간접적으로 PPARγ 활성을 줄임을 확인하였다. 즉, AQ 는 PPARγ, PGC1α 단백질의 세포질로의 이동 촉진을 통해 PPARγ 활성화에 의해 유도되는 지방세포분화를 강력하게 억제하는 반면, CQ 는 PPARγ 활성화 촉진인자인 PGC1α 단백질의 세포질로의 이동 촉진에 의해 PPARγ 매개 지방세포분화를 AQ 보다는 약하게 억제함을 확인하였다. 결론적으로 말라리아 치료제로 사용되는 AQ, CQ 를 지방세포분화 조절제로 활용 가능함을 제시하였다.