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Research Article
Protective Effect of Baicalein on oxLDL-induced Oxidative Stress and Inflammation Injury in Endothelial Cell

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Abstract

Background and Objective: There is consensus that oxidized LDL (oxLDL) play an important role in cardiovascular diseases. The aim of this study was to investigate the suppressive effects of baicalein on oxLDL-induced inflammation and oxidative stress in human microvascular endothelial cells (HMEC-1) cultures. **Materials and Methods:** The HMEC-1 were treated with 200 μg mL^{-1} oxLDL in the presence of baicalein (0-20 μM). The cell viability were detected by MTT assay, Reactive Oxygen Species (ROS) were detected using the fluorescent probe DCFH2-DA, inflammatory cytokines were assessed by enzyme-linked immunosorbent assay (ELISA) and NF-κB activity was detected using a NF-κB p65 assay kit. **Results:** The results showed that baicalein treatment induced the increase of cell viability in oxLDL treatment groups. More, ROS levels decreased by baicalein treatment. The inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) also reduced by baicalein treatment. Further, baicalein inhibits oxLDL-induced NF-κB level. **Conclusion:** This data suggested that baicalein might abolish inflammation and oxidative stress in oxLDL-treated HMEC-1 via NF-κB.

Key words: Baicalein, oxidized LDL, oxidative stress, inflammation, interleukin 6, tumor necrosis factor, NF-κB, human microvascular endothelial cells

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Baicalein is flavonoid isolated from *S. baicalensis*. Baicalein has been widely used as an antioxidant in China[1]. Baicalein has also been applied for anti-cancer, anti-inflammation in cell cultures and animal models, understanding of the molecular mechanisms still limited[2-4].

The oxLDL enhanced oxidative and inflammation stress to the vascular endothelium[5,6]. The oxLDL stimulated adhesion molecules overexpression on the endothelial cells surface, it could cumulative adherent monocytes to the arterial wall. Then adherent monocytes differentiate into macrophages, triggering the cholesterol accumulation and the foam cell formation[7].

Endothelial dysfunction has been demonstrated to be an important factor in the cardiovascular diseases development[8-10]. Moreover, endothelial dysfunction could induce cardiovascular inflammation and platelet adhesion[11,12]. Previous study revealed that oxLDL induces endothelial dysfunction[13-15]. The oxLDL level was correlated with the inflammatory response[6,16]. Nevertheless, the underlying mechanisms of the suppressive effects of baicalein on oxLDL-induced HMEC-1 injury remain unknown.

In this study, HMEC-1 were treated with oxLDL and baicalein (Fig. 1) to determine whether oxLDL-caused inflammation and oxidative stress could be suppressed by baicalein treatment.

MATERIALS AND METHODS

Compounds: Baicalein was obtained from Selleck Chemicals (Houston, TX, USA). Oxidized LDL was obtained from BioSun (Shanghai BioSun, Shanghai, China).

Cell culture: The HMEC-1 were cultured in MCDB 131 medium containing with 10% fetal bovine serum. The HMEC-1 were cultured at 37°C in humidified air containing 5% CO_2. The cells were treated with oxLDL (200 μg mL^{-1}) in the presence of baicalein (0-20 μM) for 24 h. Then biomarkers in the cells and supernatant were detected.

Cell viability determination: The HMEC-1 (3×10^4 cells mL^{-1}) were seeded in 96-well plates incubated overnight. Subsequently, 100 μL culture media containing oxLDL (200 μg mL^{-1}) in the presence of baicalein (0-20 μM) were added into the wells at 37°C for 24 h. Plain medium containing 0.5% v/v DMSO were used to the vehicle group. The cytotoxicity was determined by MTT experiment as described in an earlier study[17]. Then, 100 μL of MTT was added to the medium for 4 h. Subsequently, 150 μL of isopropanol was added into medium for 15 min and the absorbance at 570 nm was detected by a microplate reader. Results were expressed as the relative OD (570 nm wavelength) ratio in comparison with the vehicle group: OD 570 of sample group/OD 570 of vehicle group×100%.

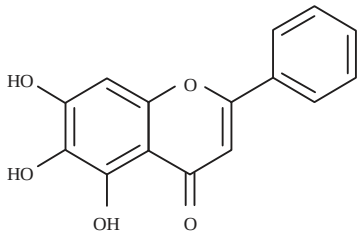

Fig. 1: Chemical structure of baicalein

Measurement of Reactive Oxygen Species (ROS): Intracellular ROS production was measured using fluorescent probes DCFH2-DA as previously described[18]. After HMEC-1 cells treatment with oxLDL (200 μg mL^{-1}) in the presence of baicalein (0-20 μM), the DCFH2-DA were loaded for 20 min at 37°C in the dark. The fluorescence corresponding to intracellular ROS levels was monitored at 488 nm (excitation) and 519 nm (emission) using a confocal fluorescence microscope (Nikon Instruments Inc. (Melville, NY, USA)). The intensity of fluorescence was determined in three independent experiments.

Inflammatory cytokines determination: After oxLDL (200 μg mL^{-1}) in the presence of baicalein (0-20 μM) treatment on HMEC-1 for 24 h, the supernatants of cells were collected. The levels of IL-6 and TNF-α in HMEC-1 were quantified by enzyme-linked immunosorbent assay (ELISA) R and D systems, Minneapolis, MN, USA) by the microplate reader[19].

Measurement of NF-κB production: After oxLDL (200 μg mL^{-1}) in the presence of baicalein (0-20 μM) treatment on HMEC-1 for 24 h, nuclear extracts from treated cells were prepared by the Nuclear Extract Kit (ab113474, Abcam). The NF-κB activity was detected using a NF-κB p65 assay kit (SN368, Beyotime Institute of Biotechnology, China) according to the instruction.

Statistical analysis: Differences between treatments were assessed by one-way ANOVA followed by a Tukey test (Bartlett test $p>0.05$) to compare mean of treatments with controls. Results are given as mean±standard error of mean.

RESULTS

Baicalein protects oxLDL reduced HMEC-1 viability: The MTT assay demonstrated that 0, 5, 10 and 20 μM baicalein to select the optimal dose to avoid cytotoxicity. Incubation with 5-20 μM baicalein for 24-48 h did not affect HMEC-1 cell viability (Fig. 2a, b), then 20 μM baicalein was used in further experiments. Figure 2c shows that, baicalein treatment induced the increase of cell viability in oxLDL treatment group.

Baicalein inhibits oxLDL induced intracellular oxidative stress in HMEC-1: To determine the oxidative stress in HMEC-1, intracellular ROS were detected. The DCFH2-DA staining showed that the ROS level reduced in a dose-dependent manner in baicalein treatment groups when compared with the oxLDL group (Fig. 3).

Baicalein inhibits oxLDL induced the levels of inflammatory cytokines: To investigate whether baicalein could reverse the increase inflammatory respond induced by oxLDL, IL-6 and TNF-α expression were detected by ELISA. Figure 4 and 5 shows the baicalein induced a dose-dependent decline of IL-6 and TNF-α expression in oxLDL group.

Baicalein inhibits oxLDL induced NF-κB level: Because the NF-κB activity is mediators of oxidative stress-induced endothelial injury and protective effects, then we detected the connection between the effects of baicalein and this signaling. Figure 6 shows that, NF-κB level was up-regulated in oxLDL-treated HMEC-1 cells. However, NF-κB levels is restored by baicalein treatment.

DISCUSSION

Oxidized low density lipoprotein (oxLDL) is recently identified as a key risk factor in cardiovascular diseases[20,21]. Baicalein has been obtained to elevate the apoptosis of cancer cells[22]. Baicalein also significantly inhibit the migration, adhesion and invasion of cancer cells[23,24]. In this study, it is reported that baicalein could suppress ROS, IL-6 and TNF-α generation, implying that the oxidative stress and inflammation respond via NF-κB signal may be the fundamental progress in oxLDL induced endothelial injuries.

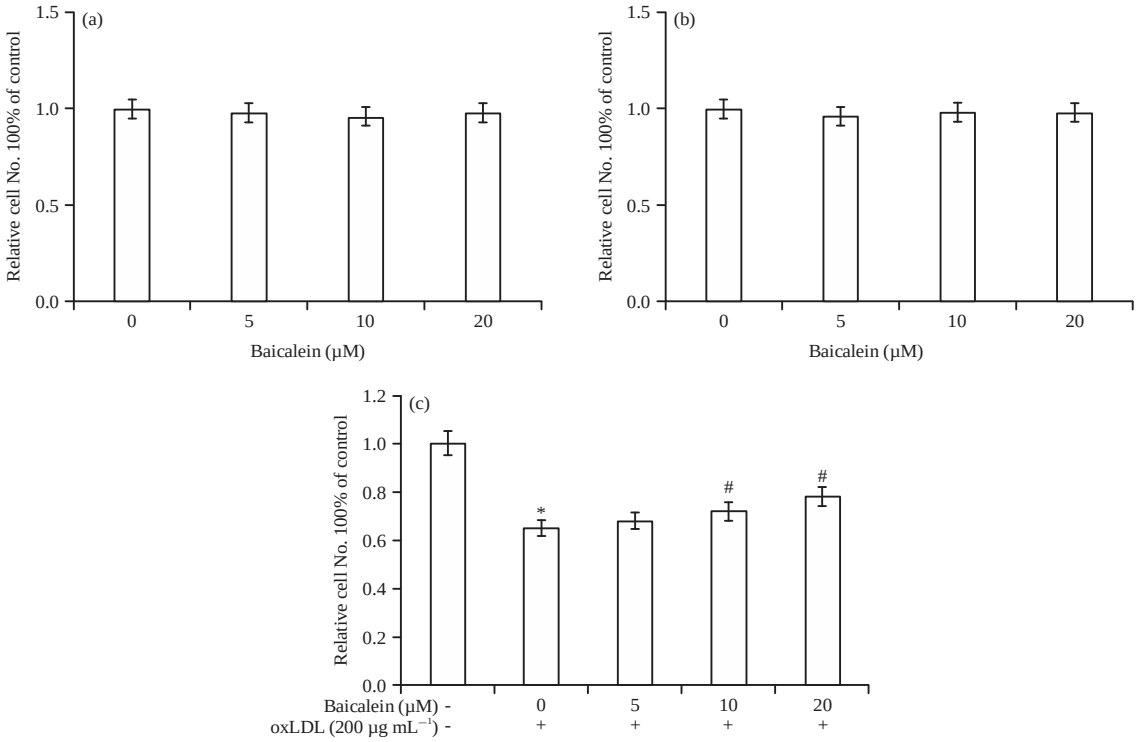

Fig. 2(a-c): Effect of baicalein on oxLDL reduces HMEC-1 cells growth was detected by MTT assay for (a) 24 h and (b) 48 h and (c) HMEC-1 cells were treated with different concentrations of baicalein for 24 h. Baicalein significantly attenuated the oxLDL reduces HMEC-1 cells growth in a dose-manner. Data are expressed as Mean±SEM, *$p<0.05$, compared to the control group (DMSO treatment), #$p<0.05$, compared to the oxLDL group

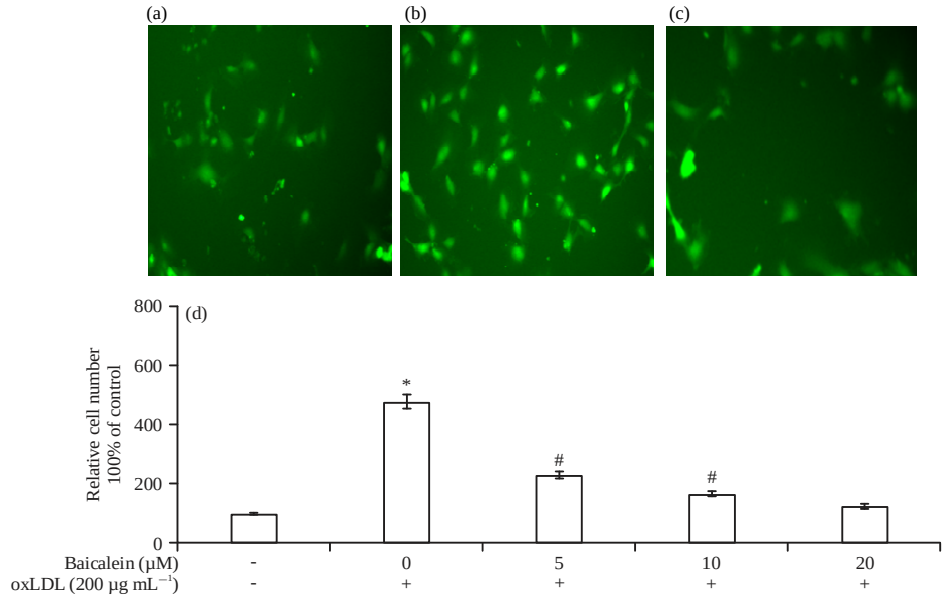

Fig. 3(a-d): Effect of baicalein on oxLDL induced Reactive Oxygen Species (ROS) production in HMEC-1, (a) Control, (b) oxLDL (200 μg mL^{-1}), (c) oxLDL (200 μg mL^{-1})+baicalein (20 μM) and (d) Data is expressed as Mean±SEM, *p<0.05, compared to the control group (DMSO treatment), #p<0.05, compared to the oxLDL group

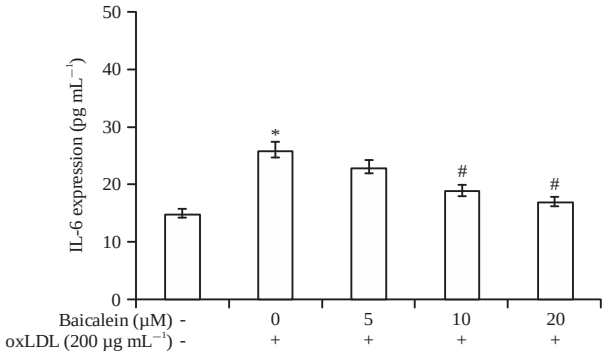

Fig. 4: Effect of baicalein on oxLDL induced interleukin 6 (IL-6) production in HMEC-1. Data are expressed as Mean±SEM, *p<0.05, compared to the control group (DMSO treatment), #p<0.05, compared to the oxLDL group

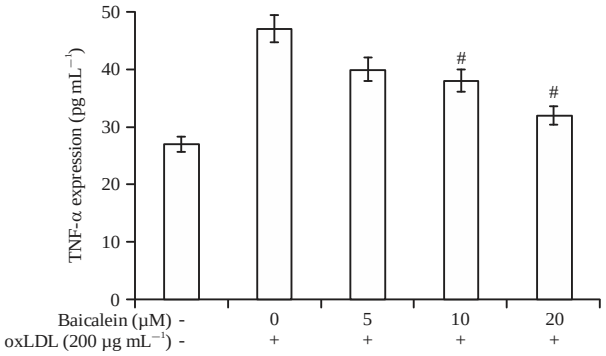

Fig. 5: Effect of baicalein on oxLDL induced tumor necrosis factor α (TNF-α) production in HMEC-1. Data are expressed as Mean±SEM, *p<0.05, compared to the control group (DMSO treatment), #p<0.05, compared to the oxLDL group

In order to detect the cytotoxicity of baicalein on HMEC-1 cells, the MTT assay was conducted to reveal the cell viability. The data indicated that baicalein at 20 μM shown to be nontoxic to HMEC-1. Furthermore, baicalein reversed oxLDL reduces HMEC-1 viability in accordance with previous research conclusions[4,25].

Vascular cells dysfunction caused by oxLDL increased ROS generation and decreased cell viability, thereby induced endothelial injury[26,27]. It is demonstrated that baicalein inhibited ROS formation (Fig. 3). Therefore, it is indicated that baicalein reduces ox LDL-induced oxidative stress.

Inflammatory response is also considered as a potential molecular mechanism on oxLDL stimulated cardiovascular injury[28]. The upregulation of IL-6 expression by oxLDL appears to be the result of oxLDL induced oxidative stress in the cell[29]. In this study, IL-6level reduced in baicalein treatment group. So, baicalein could reduce oxLDL induced cellular oxidative stress. The upregulation of TNF-α expression by oxLDL could enhance endothelial apoptosis[30]. In this study, TNF-α level reduced in baicalein treatment group. So, baicalein could reduce oxLDL induced endothelial apoptosis. Taken together, these data

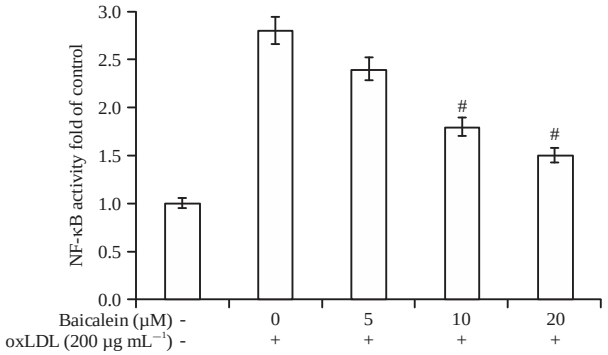

Fig. 6: Effect of baicalein on oxLDL induced NF-κB production in HMEC-1. Data are expressed as Mean±SEM. *p<0.05, compared to the control group (DMSO treatment). #p<0.05, compared to the oxLDL group

provided evidence of the baicalein suppresses proinflammatory cytokines levels in oxLDL treated HMEC-1.

The NF-κB signal is involved in the inflammatory and proliferative effects[31,32]. In the present study, baicalein decreased intracellular ROS and down regulated NF-κB level in HMEC-1. Therefore, the anti-activation of NF-κB might be necessary for the protecting effects of baicalein in HMEC-1.

CONCLUSION

In conclusion, the present data implied that baicalein may be regarded as a potential agent in the prevention of atherosclerosis. Baicalein reduced oxLDL-induces endothelial oxidative and inflammatory dysfunction via NF-κB signaling. Our report suggested that baicalein has beneficial effects on human cardiovascular diseases. More *in vivo* reports need to be use to confirm the positive effects of baicalein against endothelial cells injury.

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