Baicalin promotes cholesterol efflux by regulating the expression of SR-BI in macrophages

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Abstract. Intake of a high dosage of baicalin has previously been shown to attenuate hyperlipidemia induced by a high-fat diet. Baicalin functions as an activator of peroxisome proliferator-activated receptor-γ (PPAR-γ), which is the key regulator of reverse cholesterol transport (RCT). The present study aimed to test the hypothesis that baicalin could promote cholesterol efflux in macrophages through activating PPAR-γ. Phorbol 12-myristate 13-acetate-stimulated THP-1 cells were treated with oxidized low-density lipoprotein and (^{3}H)-cholesterol for 24 h, and the effects of baicalin on cholesterol efflux were evaluated in the presence of apolipoprotein A-1 (ApoA-1), or high-density lipoprotein subfraction 2 (HDL_2) or subfraction 3 (HDL_3). The expression levels of scavenger receptor class B type I (SR-BI), PPAR-γ and liver X receptor-α (LXRα) were detected and specific inhibitors or activators of SR-BI, PPAR-γ and LXRα were applied to investigate the mechanism. Treatment of THP-1 macrophages with baicalin significantly accelerated HDL-mediated, but not ApoA-1-mediated cholesterol efflux. However, baicalin treatment increased the expression of SR-BI at the mRNA and protein levels in a dose- and time-dependent manner, and pre-treatment with the SR-BI inhibitor BLT-1 and SR-BI small interfering RNA significantly inhibited baicalin-induced cholesterol efflux. Furthermore, baicalin increased the expression of PPAR-γ and LXRα, and the application of specific agonists and inhibitors of PPAR-γ and LXRα changed the expression of SR-BI, as well as cholesterol efflux. It may be concluded that baicalin induced cholesterol efflux from THP-1 macrophages via the PPAR-γ/LXRα/SR-BI pathway.

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Key words: cholesterol efflux, baicalin, scavenger receptor class B type I, peroxisome proliferator-activated receptor-γ, liver X receptor-α

Introduction

It is widely accepted that cholesterol is vital for survival for due to its role in maintaining the fluidity and elasticity of cellular membranes (1,2) and participating the biosynthesis of steroid hormones (3). However, an anomalous increase of cholesterol in the blood, as well as an imbalance of cholesterol metabolism, is correlated with an increased risk of atherosclerosis (4,5). The accumulation of cholesterol promotes the transformation of macrophages into lipid-laden foam cells, which is an important hallmark of atherosclerosis. High-density lipoprotein (HDL) plays a role in preventing the progression of atherosclerotic plaque through its ability to transport cholesterol from macrophages in the vessels to the liver, which is known as reverse cholesterol transport (RCT) (6).

Cholesterol efflux, by which cholesterol-loaded macrophages within the vessel wall secrete cholesterol outside cells, is understood to be a major process in RCT (7). Scavenger receptor class B type I (SR-BI), an 82-kDa glycoprotein comprising 509 amino acids, is a member of the CD36 superfamily of proteins. SR-BI has been identified as the HDL receptor which mediates the uptake of cholesterol from HDL to cells and cholesterol efflux from the cell to HDL particles (8). HDL, as well as apolipoprotein A-1 (ApoA-1), the principal apolipoprotein of HDL, plays an important role in RCT. As extracellular acceptors, HDL and ApoA-1 determine the efficiencies of RCT and cholesterol efflux (7).

The peroxisome proliferator-activated receptor-γ (PPAR-γ) is a member of a group of nuclear receptor proteins, which is involved in the regulation of cellular differentiation, development and metabolism (9). Moreover, recent studies have provided evidence that PPAR-γ is highly expressed in macrophages, as well as in the foam cells of atherosclerotic plaques (10) and mediates the expression of a cascade of genes involved in cholesterol efflux (9,11).

Baicalin is a flavonoid glycoside extracted from the dry roots of the traditional Chinese drug *Scutellaria baicalensis*,

which has been extensively used for thousands of years. Baicalin has also been purified and used in infectious and inflammatory diseases for its multiple biological properties, including anti-inflammatory (12), antioxidant (13), antimicrobial (14) and anticancer (15) activities. Furthermore, baicalin has been demonstrated to function as an activator of PPAR-γ, and thus modulate the erythroid differentiation of blood hematopoietic stem cells (16). Therefore, in view of the effect of baicalin on PPAR-γ, the aim of the present study was to investigate whether baicalin could also promote cholesterol efflux in macrophages through the activation of PPAR-γ.

Materials and methods

Reagents. Baicalin, BLT-1, GW9662, rosiglitazone, geranylgeranyl pyrophosphate (GGPP) and GW3965 were purchased from Sigma-Aldrich (Merck Millipore, Darmstadt, Germany). Rabbit polyclonal antibodies to GAPDH (cat. no. ab9485), SR-BI (cat. no. ab106572), PPAR-γ (cat. no. ab45036) and liver X receptor-α (LXRα; cat. no. ab3585) were purchased from Abcam (Cambridge, UK). SR-BI small interfering RNA (siRNA) was from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA).

Cell culture. THP-1 cells were purchased from American Type Culture Collection (Manassas, VA, USA). THP-1 cells were maintained in RPMI-1640 (Hyclone; GE Healthcare Life Sciences, Logan, UT, USA) supplemented with 10% fetal bovine serum (Hyclone), streptomycin (100 mg/ml) and penicillin (100 U/ml) at 37˚C in a humidified atmosphere with 5% CO_2. In order to stimulate the differentiation of cells into macrophages, THP-1 cells in 6-well culture dishes were treated with 160 nmol/ml phorbol 12-myristate 13-acetate for 72 h.

Cholesterol efflux assay. Following differentiation of the cells into macrophages, 50 mg/l oxidized low-density lipoprotein (oxLDL; Biomedical Technologies, Inc., Stoughton, MA, USA) and (^{3}H)-cholesterol (1.0 μCi/ml; New England Nuclear Corp., Boston, MA, USA) were added to the THP-1 cell medium for another 24 h. The cholesterol-loaded macrophages were then exposed to baicalin of different concentrations (0, 2, 10 and 50 μM) for 48 h, or at a concentration of 50 μM for different times (0, 6, 12, 24 and 48 h) in the presence of ApoA-1 (10 μg/ml; Sigma-Aldrich), HDL subfraction 2 (HDL_2; 50 μg/ml) or HDL subfraction 3 (HDL_3; 50 μg/ml) (both purchased from USBiological, Swampscott, MA, USA. To confirm the effect of the PPAR-γ signaling pathway, the cells were pre-treated with PPAR-γ antagonist GW9662 (20 μM) for 2 h before stimulation with baicalin, or treated with PPAR-γ agonist rosiglitazone (1 μM) for 12 h. To confirm the effect of the LXRα signaling pathway, the cells were pre-treated with LXRα antagonist GGPP (5 μM) for 2 h before stimulation by baicalin, or treated with LXRα agonist GW3965 (5 μM) for 12 h. For analysis of cholesterol efflux, the medium and the THP-1 macrophages were collected, respectively, and the radioactive content was determined by liquid scintillation (Beckman LS6500; Beckman Coulter, Inc., Fullerton, CA, USA). The percentage of cholesterol efflux was calculated by dividing the radioactive content in the medium by the sum of the radioactive content in the medium and in the cells (17).

Western blot analysis. Radioimmunoprecipitation assay buffer and phenylmethanesulfonyl fluoride (Beyotime Institute of Biotechnology, Haimen, China) were used to extract the protein from the THP-1 macrophages following the various treatments. The bicinchoninic acid method was used to measure the protein concentration of the lysates. Moderate quantities (5-10 μl) of protein lysates were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis at 90 V. The proteins were transferred to nitrocellulose membranes (EMD Millipore, Billerica, MA, USA), which were then blocked with 5% non-fat milk for 1 h at room temperature and incubated with primary antibodies against GAPDH (1:1,000), SR-BI (1:500), PPAR-γ (1:1,000) and LRXα (1:500) overnight at 4˚C. Finally, following incubation with goat anti-rabbit secondary antibody (cat. no. ZDE-5209; for GAPDH, 1:10,000; for SR-BI, 1:2,000; for PPAR-γ, 1:5,000; for LXR, 1:1,000; ZSGB-Bio, Beijing, China) for an additional 2 h at room temperature, the antigen-antibody complex was detected using an electrochemiluminescence detection system (Immobilon Western Chemiluminescent HRP Substrate; EMD Millipore). The blots were quantified using Adobe Photoshop CS5 (Adobe Systems, Inc., San Jose, CA, USA)

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Following treatment, total RNA was extracted from the THP-1 macrophages using TranZol Up (Transgen Biotech, Beijing, China). Then, RNA samples were treated with RNase-free DNase (Transgen Biotech). Following extraction, 1 μg mRNA was reversely transcribed using a First Strand cDNA Synthesis kit (Fermentas; Thermo Fisher Scientific, Inc., Waltham, MA, USA). After that, total cDNA was amplified using the FastStart Universal SYBR Green Master (ROX) mix (Roche Diagnostics, Basel, Switzerland) in the Light Cycler real-time PCR detection system (Roche Diagnostics) for 40 cycles at 95˚C for 10 sec, 60˚C for 20 sec and 72˚C for 30 sec. 18S was chosen as the reference gene and the primer sequences for qPCR analyses were as follows: 18S, forward primer: 5'-CTTAGTTGGTGGAGCGATTTG-3', reverse primer: 5'-GCTGAACGCCACTTGTCC-3' (17). SR-BI, forward primer 5'-TCCTCACTTCCTCAACGCTG-3' and reverse primer 5'-TCCCAGTTTGTCCAATGCC-3' (18). Melting curves were assessed to confirm the specificity of the products generated for each set of primers. The $2^{-\Delta\Delta Cq}$ comparative method was then used to normalize the relative levels of gene expression (19).

Statistical analysis. SPSS statistical analytical software, version 18.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. The normally distributed data were analyzed by one-way analysis of variance and the nonparametric variables were analyzed by Mann-Whitney U test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Baicalin promotes cholesterol efflux to ApoA-1, HDL_2 and HDL_3 in THP-1 macrophages. To determine the effect that baicalin had on cholesterol efflux in THP-1 macrophages, THP-1 cells were first exposed to oxLDL and (^{3}H) cholesterol for 24 h, and then the cholesterol-loaded THP-1 macrophages

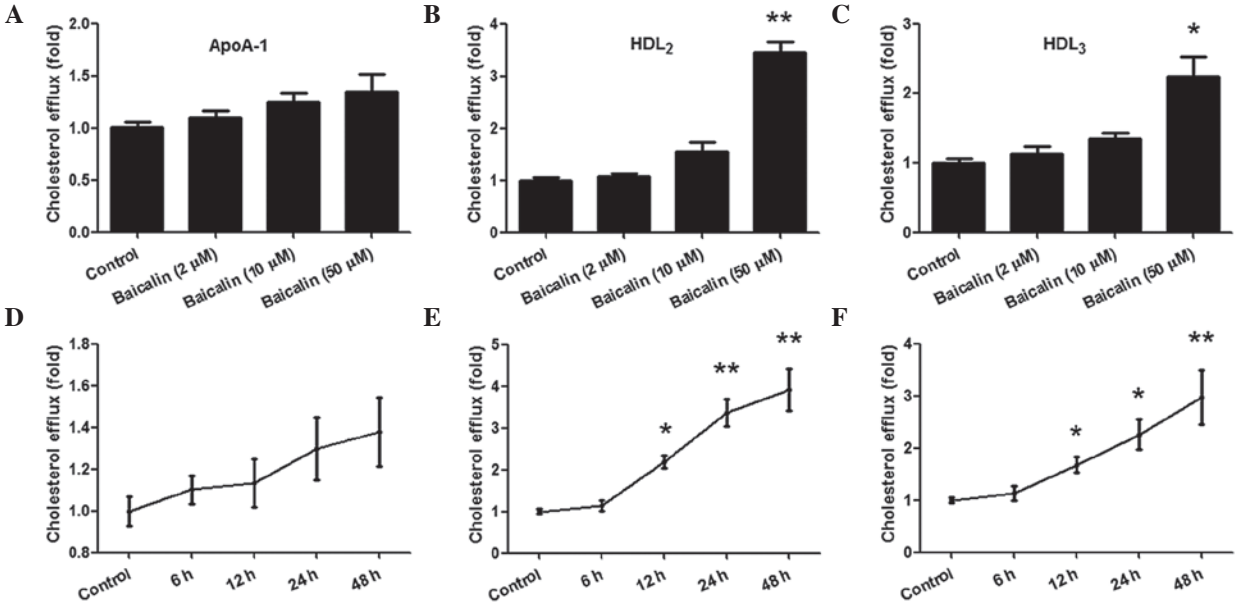

Figure 1. Effects of baicalin on cholesterol efflux in THP-1 macrophages. Following stimulation with phorbol 12-myristate 13-acetate, THP-1 macrophages were exposed to oxidised low-density lipoprotein and (^{3}H)-cholesterol for 24 h, and the effect of baicalin on cholesterol efflux was evaluated in the presence of ApoA-1, HDL_2 or HDL_3. The cells were treated with different concentrations of baicalin for 48 h, and cholesterol efflux was (A) not significantly changed in the presence of Apo-A1, but was significantly increased in a concentration-dependent manner in the presence of (B) HDL_2 or (C) HDL_3, Cells were treated with 50 μM baicalin for different time periods, and cholesterol efflux was (D) not significantly increased by ApoA-1, but was increased in a time-dependent manner in the presence of (E) HDL_2 and (F) HDL_3. *P<0.05 vs. control group; **P<0.01 vs. control group. Data shown are means ± standard error of the mean from three independent experiments in duplicate. ApoA-1, apolipoprotein A-1; HDL_2, high-density lipoprotein subfraction 2; HDL_3, high-density lipoprotein subfraction 3.

were cultured with different concentrations of baicalin (0, 2, 10 and 50 μM) for 48 h in the presence of ApoA-1, HDL_2 or HDL_3 in the medium (Fig. 1). As shown in Fig. 1A-C, baicalin at concentrations of 2 and 10 μM failed to promote cholesterol efflux to ApoA-1, HDL_2 and HDL_3. However, 50 μM baicalin significantly promoted cholesterol efflux to HDL_2 and HDL_3 by ~3.4- and 2.2-fold respectively compared with the control group (P<0.05), while this concentration of baicalin failed to promote cholesterol efflux to ApoA-1 (P>0.05). The results demonstrated that baicalin accelerated cholesterol efflux to HDL_2 and HDL_3, but not ApoA-1, when its concentration reached 50 μM.

Subsequently, 50 μM baicalin was used to stimulate the cholesterol-loaded THP-1 macrophages for various times (0, 6, 12, 24 and 48 h). As shown in Fig. 1E and F, incubation with baicalin for 6 h did not enhance cholesterol efflux to HDL_2 and HDL_3, while ≥12 h incubation significantly increased cholesterol efflux (P<0.05), which peaked at 48 h (P<0.01). However, as shown in Fig. 1D, incubation with baicalin did not increase cholesterol efflux to ApoA-1. These results indicate that baicalin is able to accelerate cholesterol efflux to HDL_2 and HDL_3, but not ApoA-1, in THP-1 macrophages in a concentration- and time-dependent manner.

Baicalin enhances the expression of SR-BI and thereby mediates cholesterol efflux in THP-1 macrophages. SR-BI has been reported to be the major mediator of cellular cholesterol efflux (20). In the present study, in order to determine whether the effect of baicalin on cholesterol efflux in THP-1 macrophages was mediated by SR-BI, the expression of SR-BI was estimated. The cells were first treated with different concentrations (0, 2, 10 and 50 μM) of baicalin for 48 h. SR-BI was detected by western blotting and RT-qPCR at the protein and mRNA levels, respectively. As shown in Fig. 2A, following stimulation with 50 μM baicalin for 48 h, the expression of SR-BI was significantly increased compared with that in the control group (P<0.01). However, although 2 and 10 μM baicalin also slightly increased the expression of SR-BI, the increases were not statistically significant. Subsequently, baicalin (50 μM) was cultured with the cells for different time periods (0, 6, 12, 24 and 48 h). As shown in Fig. 2B, the relative expression of SR-BI was significantly increased when the stimulation time reached 12 h (P<0.05), and peaked at 24-48 h (P<0.01). RT-qPCR was used to examine the mRNA level of SR-BI after the treatment, and similar results were obtained; the expression of SR-BI at the mRNA level exhibited changes similar to those of the protein with different concentrations of baicalin (Fig. 2C) and different treatment durations (Fig. 2D). These data showed that baicalin induced SR-BI production at the protein and mRNA levels in a concentration- and time-dependent manner.

To further confirm the pivotal role of SR-BI in baicalin-induced cholesterol efflux, BLT-1 (a specific inhibitor of SR-BI) and SR-BI siRNA were each used to pre-treat the cells for 2 h before baicalin was added into the cells. As shown in Fig. 2E and F, BLT-1 and SR-BI siRNA significantly inhibited baicalin-induced cholesterol efflux compared with that in the baicalin group (P<0.05). Thus, all the results above suggest that baicalin enhanced cholesterol efflux in THP-1 macrophages via SR-BI.

Baicalin-induced cholesterol efflux in THP-1 macrophages is mediated by PPAR-γ. PPAR-γ has been reported to promote

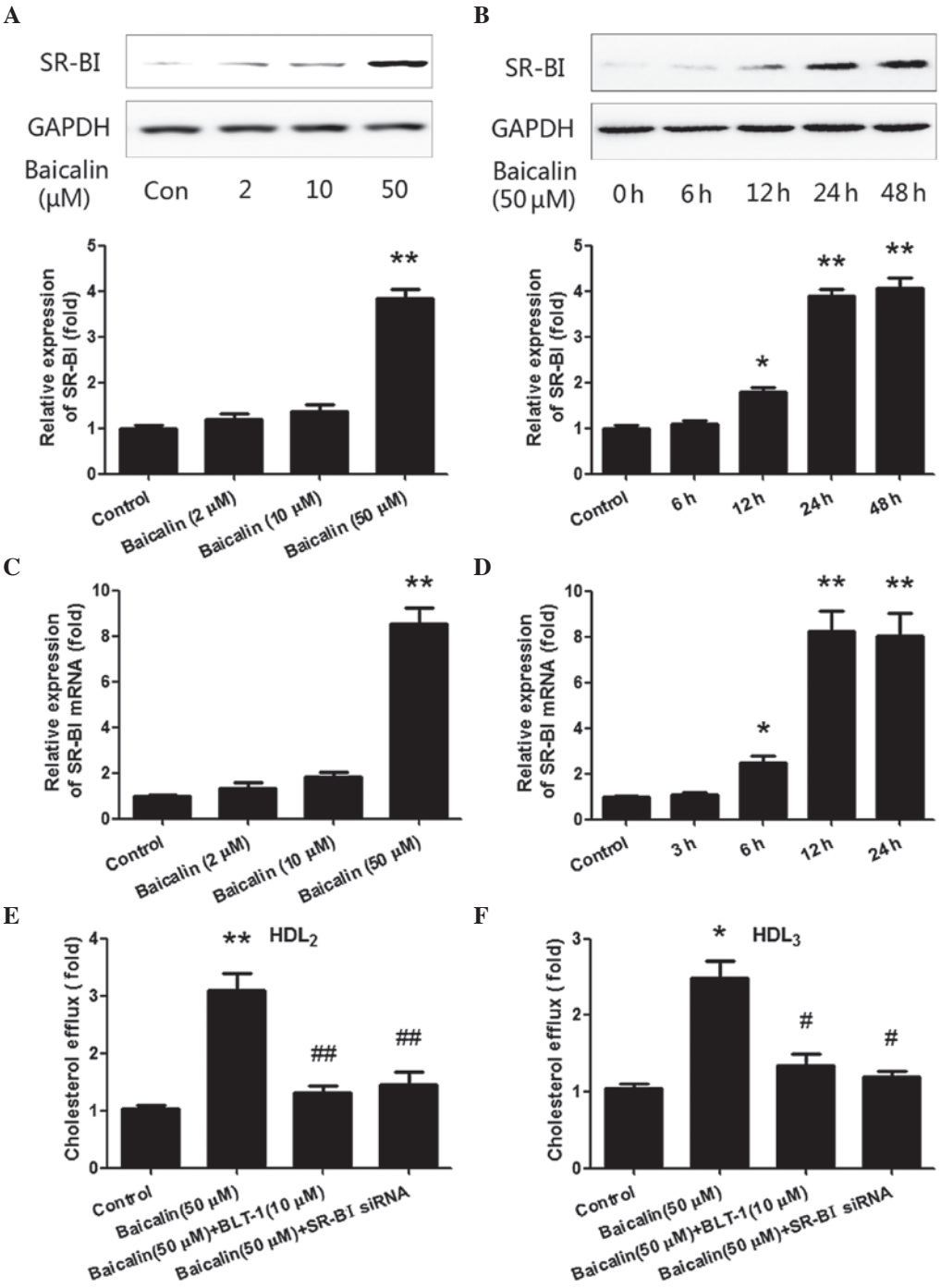

Figure 2. Baicalin upregulates SR-BI expression in THP-1 macrophages. THP-1 macrophages were stimulated with (A) different concentrations of baicalin for 48 h, and (B) 50 μM baicalin for different time periods, and western blotting was used to detect the expression of SR-BI. THP-1 macrophages were stimulated with (C) different concentrations of baicalin for 24 h, and (D) 50 μM baicalin for different time periods, and reverse transcription-quantitative polymerase chain reaction analysis was used to detect the expression of SR-BI mRNA. (E and F) Pre-incubation with SR-BI antagonist BLT-1 (10 μM) and SR-BI siRNA significantly inhibited the upregulating effect of baicalin on cholesterol efflux in the presence of (E) HDL_2 or (F) HDL_3. *P<0.05 vs. control group; **P<0.01 vs. control group; $^{\#}$P<0.05 vs. the baicalin group; $^{\#\#}$P<0.01 vs. the baicalin group. Data shown are means ± standard error of the mean from three independent experiments in duplicate. SR-BI, scavenger receptor class B type I; siRNA, small interfering RNA; HDL_2, high-density lipoprotein subfraction 2; HDL_3, high-density lipoprotein subfraction 3.

cholesterol efflux and regulate the expression of SR-BI (19,21). In order to determine whether PPAR-γ is involved in the regulation of cholesterol efflux by baicalin, the expression of PPAR-γ in THP-1 macrophages following treatment with baicalin was examined. As shown in Fig. 3A, following stimulation with baicalin (50 μM) for 3 h, the expression of PPAR-γ significantly increased (P<0.05), and a peak was reached at the 12-h time point (P<0.01). An agonist and antagonist of PPAR-γ were then respectively applied to further clarify the role of PPAR-γ. As shown in Fig. 3B, pre-treatment with PPAR-γ antagonist GW9662 (20 μM) significantly inhibited the upregulated expression of SR-BI (P<0.01) stimulated by baicalin, while treatment with the PPAR-γ agonist rosiglitazone (1 μM) significantly increased the expression of SR-BI

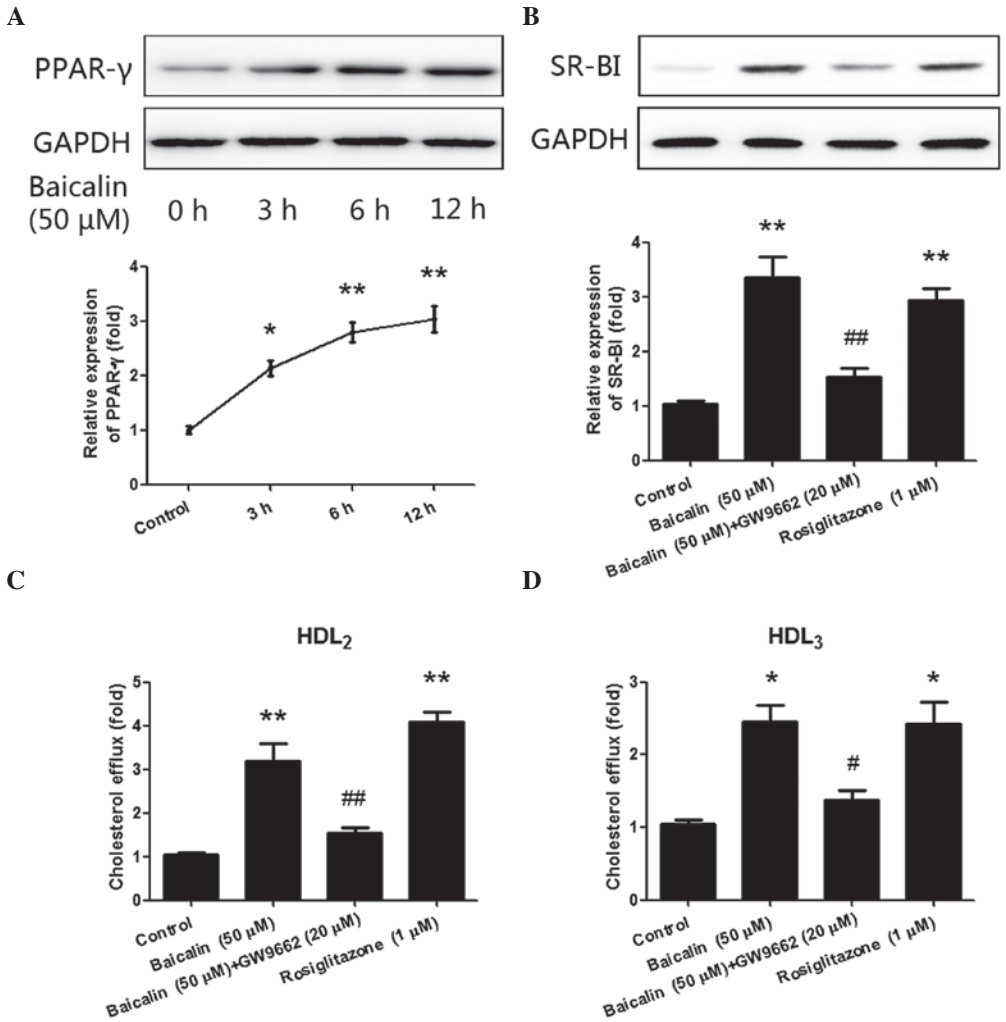

Figure 3. PPAR-γ mediated the regulation of SR-BI by baicalin. (A) THP-1 macrophages were stimulated with 50 μM baicalin for 0, 3, 6 and 12 h, and the expression of PPAR-γ was significantly increased in a time-dependent manner. (B) Pre-incubation with PPAR-γ antagonist GW9662 (20 μM) significantly inhibited the upregulating effect of baicalin on the expression of SR-BI, while PPAR-γ agonist rosiglitazone (1 μM) significantly enhanced the expression of SR-BI. (C and D) Pre-incubation with GW9662 (20 μM) significantly inhibited the upregulating effect of baicalin on cholesterol efflux, while PPAR-γ agonist rosiglitazone (1 μM) significantly increased cholesterol efflux, in the presence of (C) HDL_2 or (D) HDL_3. *P<0.05 vs. control group; **P<0.01 vs. control group; $^{\#}$P<0.05 vs. the baicalin group; $^{\#\#}$P<0.01 vs. the baicalin group. Data shown are means ± standard error of the mean from three independent experiments in duplicate. PPAR, peroxisome proliferator-activated receptor; SR-BI, scavenger receptor class B type I; HDL_2, high-density lipoprotein subfraction 2; HDL_3, high-density lipoprotein subfraction 3.

(P<0.01). Cholesterol efflux was then estimated following the treatment with GW9662 and rosiglitazone. As shown in Fig. 3C and D, cholesterol efflux was significantly inhibited following pre-incubation with PPAR-γ antagonist GW9662, but increased after incubation with PPAR-γ agonist rosiglitazone. Thus, these results suggest that baicalin-induced cholesterol efflux was mediated by PPAR-γ.

Baicalin-induced cholesterol efflux in THP-1 macrophages is mediated by LXRα. LXRα has been identified as a target gene of PPAR-γ. The binding of PPAR-γ with the peroxisome proliferator hormone response element (PPRE), which is located in the promoter region of LXRα, is reported to directly upregulate the expression of LXRα (11). PPAR-γ and LXRα are involved in a metabolic cascade that regulates the expression of downstream genes and increases cholesterol efflux (9,22). Thus, in the present study, the expression of LXRα in THP-1 macrophages was detected following the treatment with baicalin.

As shown in Fig. 4A, although the expression of LXRα only increased slightly after 3 h stimulation with baicalin (P>0.05), it significantly increased after stimulation with baicalin (50 μM) for 6 h (P<0.05), and reached a peak at the 12-h time point (P<0.01). Subsequently, an agonist and antagonist of LXRα were also respectively applied to further confirm the role of LXRα. As shown in Fig. 4B, pre-treatment with the LXRα antagonist GGPP (5 μM) significantly inhibited the upregulating effect of baicalin on the expression of SR-BI (P<0.01), while LXRα agonist GW3965 (5 μM) significantly increased the expression of SR-BI (P<0.01). Finally, the effects of GGPP pre-treatment and GW3965 treatment on cholesterol efflux were also determined. As shown in Fig. 4C and D, cholesterol efflux was also significantly inhibited following pre-incubation with GGPP, but elevated following incubation with GW3965. The results above indicate that the PPAR-γ/LXRα pathway plays a pivotal role in cholesterol efflux, and baicalin-accelerated cholesterol efflux is mediated by the PPAR-γ/LXRα pathway in THP-1 macrophages.

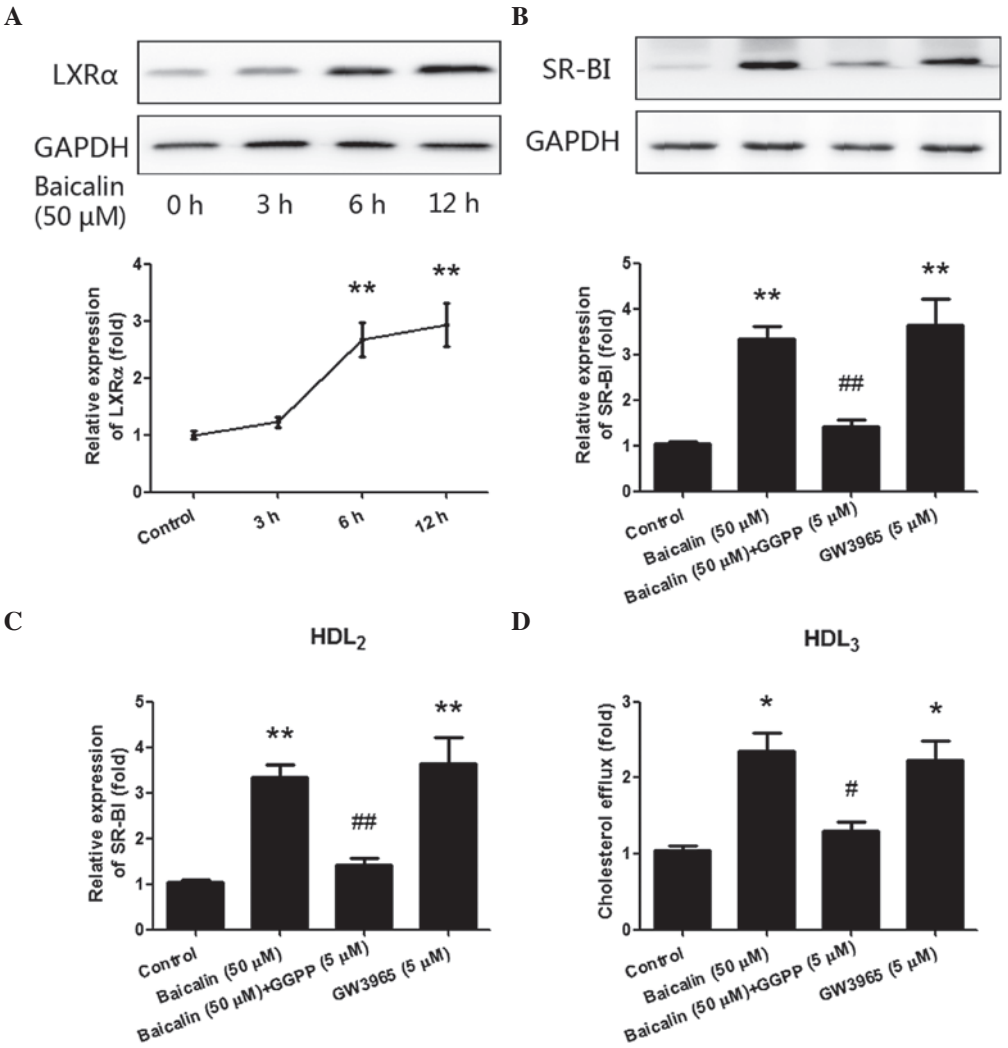

Figure 4. LXRα mediated the regulation of SR-BI by baicalin. (A) THP-1 macrophages were stimulated with 50 μM of baicalin for 0, 3, 6 and 12 h, and the expression of LXRα was significantly increased in a time-dependent manner. (B) Pre-incubation with LXRα antagonist GGPP (5 μM) significantly inhibited the upregulating effect of baicalin on the expression of SR-BI, while LXRα agonist GW3965 (5 μM) significantly increased the expression of SR-BI. (C and D) Pre-incubation with GGPP (5 μM) significantly inhibited the upregulating effect of baicalin on cholesterol efflux, while GW3965 (5 μM) significantly increased cholesterol efflux in the presence of (C) HDL_2 or (D) HDL_3. *P<0.05 vs. control group; **P<0.01 vs. control group; $^{\#}$P<0.05 vs. the baicalin group; $^{\#\#}$P<0.01 vs. the baicalin group. Data shown are means ± standard error of the mean from three independent experiments in duplicate. LXR, liver receptor X; SR-BI, scavenger receptor class B type I; HDL_2, high-density lipoprotein subfraction 2; HDL_3, high-density lipoprotein subfraction 3.

Discussion

Baicalin has been extensively studied and used for its various therapeutic potencies in inflammation (12), infections (14) and cancers (15). A recent study has shown that baicalin attenuates high-fat diet-induced obesity, liver weight, as well as hyperlipidemia and liver dysfunction (23). Intake of a high dosage of baicalin was found to significantly restore high-fat diet-induced increases of triglyceride, cholesterol and LDL levels and reduction of HDL levels (24). In addition, treatment with baicalin was also shown to decrease the atherosclerotic lesion area in the aortic sinus of $ApoE^{-/-}$ mice (25). Previous studies have discovered that PPAR-γ not only regulates the expression of its target gene LXRα, but also participates in the control of cholesterol efflux from macrophages together with LXRα. According to the study of Abbasi *et al* (16), baicalin is an activator of PPAR-γ. Thus, based on the results above, it may be speculated that baicalin could affect cholesterol metabolism through the PPAR-γ pathway.

RCT has been proposed to transport cholesterol from vessel walls to the liver and intestine for excretion or recycling, by which RCT prevents the development of atherosclerosis (26). RCT is a HDL- and ApoA-1-mediated process that involves complicated steps, beginning with the efflux of free cholesterol from peripheral tissues to the extracellular lipoprotein acceptors (17). Cholesterol efflux from cells to HDL and ApoA-1 has been widely accepted to be mediated by members of the ATP-binding cassette (ABC) transporter family, particularly ABC transporter A1 (ABCA1) (17). Studies have also demonstrated that SR-BI plays a critical role in promoting cholesterol efflux and preventing cholesterol accumulation in macrophages (27,28). A study by Jian *et al* suggested that SR-BI in cultured cells increased the rate of cholesterol efflux from the cell to HDL particles, but not to lipid-free ApoA-1 (29). Liadaki *et al* found that the high affinity binding of rHDL to SR-BI was due to the direct association of ApoA-1 with SR-BI; however, the authors failed to establish direct binding of SR-BI to the lipid-free ApoA-1 (30).

In the present study, the effects of baicalin on the cholesterol efflux of macrophages in the presence of different mediators were investigated. The results demonstrated that baicalin accelerated cholesterol efflux from THP-1 macrophages in a concentration- and time-dependent manner when the mediator was HDL_2 or HDL_3. However, when the mediator was changed to ApoA-1, the cholesterol efflux-promoting effect of baicalin disappeared. Therefore, it was speculated that the promoting effect of baicalin on cholesterol efflux might depend on the activation of SR-BI. Then, in order to confirm this hypothesis, macrophages were treated with different concentrations of baicalin for different time periods. SR-BI was detected by western blot analysis and RT-qPCR at protein and mRNA levels, respectively, and the results showed that baicalin induced SR-BI production in a concentration- and time-dependent manner. Following that, to further confirm the role of SR-BI, BLT-1 and SR-BI siRNA were used to pre-treat the cells. The results again demonstrated that both agents significantly inhibited baicalin-induced cholesterol efflux compared with that in the baicalin group. Thus, these results suggest that baicalin increased cholesterol efflux via SR-BI.

PPAR-γ and LXRα are considered to be two key nuclear receptors that are crucial in the process of RCT. PPAR-γ upregulates the expression of LXRα, either by directly binding to the PPRE within the promoter region of LXRα (31), or by other indirect pathways (11). Previous studies have demonstrated that the PPAR-γ/LXRα pathway is not only upstream of ABCA1, but also has the ability to regulate the expression of SR-BI. In a study by Tang *et al*, it was found that PPAR-A downregulated the expression of ABCA1, ABCG1 and SR-B1, but specific activation of LXRα with its agonist significantly attenuated these reductions in expression levels (32). In a study by Ma *et al* (33), it was demonstrated that treatment with LXR agonist T0901317 substantially increased the mRNA and protein expression levels of ABCA1, ABCG1 and SR-BI, and further regulated HDL- and ApoA-1-mediated cholesterol efflux. However, silencing of LXRα significantly reduced the protein levels of ABCA1, ABCG1 and SR-BI in human macrophages. A study by Kämmerer *et al* demonstrated that 13-hydroxy linoleic acid increased the expression levels of ABCA1, ABCG1 and SR-BI and stimulated cholesterol efflux in macrophages via the PPAR-γ/LXRα pathway (34). In the present study, using antagonists and agonists of PPAR-γ and LXRα, the pivotal role of the PPAR-γ/LXRα pathway in cholesterol efflux was confirmed, and it was clarified that baicalin-accelerated cholesterol efflux was mediated by PPAR-γ/LXRα pathway in THP-1 macrophages.

In conclusion, the results of the present study indicated that baicalin promotes the expression of SR-BI, and induces cholesterol efflux in macrophages through a PPAR-γ/LXRα/SR-BI signaling pathway. These results provide new insight into the effect of baicalin on cholesterol efflux, which might be a new approach for use in the management of cardiovascular diseases.

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