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Protective effects of Dioscin on TNF-α-induced collagen-induced arthritis rat fibroblast-like synoviocytes involves in regulating the LTB4/BLT pathway

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Abstract: Background and Objective: LTB4 has been shown to be involved in rheumatoid arthritis (RA) pathogenesis. The effect of Dioscin(Dio) on the LTB4 pathway of RA have not been reported yet. This study aimed at further exploring whether Dioscin's effects on TNF-α induced collagen-induced arthritis (CIA) rat fibroblast-like synoviocytes (FLS) connected with the LTB4 and its receptor pathway. **Materials & Methods:** In this experiment, control group, TNF-α group, and different concentrations of Dioscin groups were established. Cell viability was evaluated using MTT assay. The levels of LTB4 in the samples of above groups were measured using ELISA. The mRNA expression levels of LTA4H, BLT1, and BLT2 were detected by quantitative real time PCR, while the expression level of LTA4H proteins were detected using western blot. The distribution of LTA4H was assessed by immunofluorescence assay. Results: the LTB4 level of TNF-α group in sample supernatant was higher than both control group and Dioscin groups with decreased LTB4 levels ($p< 0.05$). Compared with the control group, the expression of LTA4H was significantly increased in TNF-α group ($p < 0.05$), whereas LTA4H expressions were significantly decreased in all Dioscin groups when compared to TNF-α group ($p < 0.05$). The mRNA expressions of BLT1 and BLT2 were markedly higher in TNF-α group than those in control group while Dioscin treatment significantly inhibited the increased expressions of BLT1 and BLT2 induced by TNF-α ($p < 0.05$). **Conclusions:** These results firstly demonstrate that the protective effect of Dioscin on TNF-α induced FLS may involve in its reducing LTB4 production by down-regulating LTA4H expression, and may inhibit its downstream pathway by decreasing LTB4 receptors levels. This findings suggest that dioscin produces a potential therapeutic effects for RA via its influencing LTA4H/LTB4/BLT pathway.

Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease, and its primary pathological manifestations include chronic synovial membrane inflammation or proliferation and joint erosion (Tu *et al.*, 2012; Zheng *et al.*, 2019). Abnormal hyperplasia of the synovial membrane is associated with apoptosis and abnormal proliferation of synovial cells, among which fibroblast-like synoviocytes (FLS) are the main cells involved in pannus formation and are actively involved in the progression of RA due to their abnormal apoptosis (Bartok and Firestein, 2010; Bottini and Firestein, 2013). Following stimulation by pathological factors, FLS release various cytokines that cause joint destruction (Friday and Fox, 2016). Proliferative FLS can secrete a large number of inflammatory cytokines, further causing FLS hyperproliferation and continuous inflammatory response. Up to now, the exact pathogenesis of RA has not yet been described, and there is no specific cure for it (Wu *et al.*, 2014; Li *et al.*, 2020).

As metabolic products of eicosanoids, leukotrienes (LTs) are a family of strong proinflammatory lipid mediators, which were discovered in the 1970s (Samuelsson, 1983). cPLA2 releases arachidonic acid (AA) from the cell membrane. AA is oxidized into 5-hydroperoxy-eicosatetraenoic acid, followed by transformation into the unstable epoxide leukotriene

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A4 (LTA4). LTA4 can be either metabolized into LTB4 (by LTA4 hydrolase (LTA4H)) or into the cysteinyl-leukotriene LTC4 (by LTC4 synthase (LTC4S)), which further degrades into LTD4 and LTE4.

Growing evidence indicates that dysfunction of LT synthesizing enzymes and/or receptors play a pivotal role in RA pathogenesis (Wu *et al.*, 2014). LTB4 is present at high levels in the serum and synovial fluid of RA patients (Elmgreen *et al.*, 1987). As a potent chemotactic mediator, LTB4 may induce most of the RA symptoms through the recruitment of leukocytes by initiating, coordinating, sustaining, and amplifying the inflammatory response (Yousefi *et al.*, 2014). By coupling to BLT1 and BLT2, LTB4 accelerates the neutrophil-dependent increase in microvascular permeability (Yang *et al.*, 2008; Yokomizo, 2015; Guo *et al.*, 2016). Several studies have indicated that the blockade of LTB4 and its high-affinity receptor, BLT1, dramatically inhibits arthritis in an animal model (Zhan *et al.*, 2016; Wei *et al.*, 2017). These data suggested that LTB4 and BLT1 may contribute to the pathogenesis of human RA (Miyabe *et al.*, 2017). In addition, Bi *et al.* (2017) demonstrated that LTB4 increases the levels of interleukin-32, IFN-γ, chemokines MCP-1, and MIP-1α in synovial cells and facilitates synovial cell apoptosis. Despite such progress so far, few systemic and integrated studies have been employed to explore the cause of abnormal levels of LTB4 or AA metabolites in synovial fluid and serum of patients with active RA (Zhan *et al.*, 2016; Wei *et al.*, 2017). Moreover, LTB4 receptor dysfunction in RA pathogenesis is not fully clear.

Eicosanoids (LTB4, etc.) are responsible for the progressive destruction of bone and cartilage in RA disease, so it is urgent to develop these novel compounds, which target the eicosanoid pathway by repressing the pro-inflammatory eicosanoid production in RA (Hoxha, 2017). Moreover, owing to some adverse effects resulted from long-term drug therapies for RA, it is necessary to seek safe and effective alternatives suitable for long-term chronic use. Ideal therapy of RA always involves mild but simultaneous interventions of multiple targets, which is in line with the philosophy of traditional Chinese medicine (Meng *et al.*, 2015). According to Traditional Chinese Medicine, Dioscin can promote digestion and blood circulation, relax muscles and joints, treat malaria, eliminate phlegm and diuresis, and so on (Wang *et al.*, 2017). Nowadays, it is used as an important synthetic raw material of various steroid hormone drugs. Its functions, such as immunity regulation, anti-inflammation, anti-tumor, and antiplatelet aggregation, have also been revealed (Zhang and Liu, 2010; Guo *et al.*, 2013; Qu *et al.*, 2014). So far, only a few reports have been published about Dioscin treating RA (Chu *et al.*, 2012; Guo *et al.*, 2013; Qu *et al.*, 2014), which mainly focus on inflammatory cytokines, COX pathway, and NF-kB. We previously reviewed the roles of LTB4 in RA pathogenesis, suggesting its potential significance in treating RA (Wu *et al.*, 2014; Zhan *et al.*, 2016). However, Dioscin's treatment of RA by the LTB4 pathway has not been reported yet. This study aimed to investigate the therapeutic effects and mechanisms of Dioscin against RA in TNF-α induced FLS, trying to provide new experimental evidence and approach for clinical treatment of RA.

Materials and Methods

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies (Tveden-Nyborg *et al.*, 2018).

Cells, drugs, and reagents

Dioscin monomer was bought from Nanjing Spring & Autumn Biological Engineering Co., Ltd. (Nanjing, China). Tumor necrosis factor (TNF-α) was obtained from Sigma-Aldrich (St. Louis, Missouri, USA). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT), fluorescein isothiocyanate (FITC) labeled goat anti-rabbit IgG, dimethyl sulfoxide (DMSO), and phosphate-buffered saline (PBS) were obtained from Beijing Zhongshan Biological Technology Co., Ltd. (Beijing, China). Dulbecco's modified eagle medium (DMEM) culture medium was purchased in Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Fetal bovine serum (FBS) was bought from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. (Hangzhou, China). 0.25% trypsin was supplied by Gibco BRL (Grand Island, New York, USA). To prepare the cell frozen stock solution, the allocation ratio of DMSO: FBS: DMEM was equaled to 1:2:7. Antibodies for LTA4H were obtained from Santa Cruz (California, USA). TRIzol was obtained from Takara Bio (Beijing, China). GoScriptTM Reverse Transcription System was obtained from Promega (Wisconsin, USA). Rat LTB4 ELISA assay kit purchased from Beijing Xinpeng Hongye Technology Co., Ltd. (Beijing, China). All other chemicals were of the highest purity commercially available.

Cell culture and drug treatments

Collagen-induced arthritis (CIA) rat fibroblast-like synoviocytes (FLS) were purchased from Shanghai Yansheng Industrial Co., Ltd. (Shanghai, China). The cultivation, resuscitation, and subculture of FLS were done following previous protocols. FLS were cultured in Dulbecco's modified Eagle's medium supplemented with 20% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin in a humidified atmosphere under 5% CO_2 at 37°C. The cell passage was within 10 passages from when the cell line was purchased. For all experiments, FLS cells were grown to 80–85% confluence, and then, stimulated with 10 ng/mL TNF-α or PBS for 24 h to induce an inflammatory response. Subsequently, different concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 μg/mL) of Dioscin were added to each well for another 24 h, and phosphate-buffered saline (PBS) was used as vehicle control in all experiments.

Cell viability assay

Cell viability was assessed by a modified MTT assay as described in our previous study (Hong *et al.*, 2015). Cells (1 × 105 cells/mL) were seeded in 96-well plates and incubated for 24 h. Then, the cells were exposed to 10 ng/mL TNF-α or PBS or 10 ng/mL TNF-α together with different concentrations of Dioscin for another 24 h. Thereafter, 10 μL of 5 mg/mL MTT in PBS was added to each well, and cells were incubated for a further 4 h at 37°C. The medium was then replaced by 100 μL of DMSO to

dissolve the formed precipitate. The optical density was measured in a microplate reader (Model 680, Bio-Rad, USA) at 490 nm. Viability (%) = (OD of experimental group−OD of blank control group)/(OD of control group −OD of blank control group) × 100%.

ELISA

Cells (1 × 105 cells/mL) were seeded in 96-well plates and incubated for 24 h. After that, the cells were exposed to 10 ng/mL TNF-α or PBS or 10 ng/mL TNF-α together with different concentrations of Dioscin for another 24 h. Then, 50 μL of the culture medium was assayed according to the LTB4 ELISA kit's manufacturer's instructions. The concentration of samples was determined according to the standards.

Quantitative-PCR (q-PCR)

RNA was extracted from cell samples using TRIzol reagent, and the synthesis of complementary DNA (cDNA) for qPCR analysis was performed using a kit with subsequent melting curve analysis, according to the manufacturer's protocol. The PCR reaction mixture (forward primer, 0.4 μL; reverse primer, 0.4 μL; cDNA, 2 μL; ddH$_2$O, 7 μL; CXR, 0.2 μL; GoTaq qPCR Master Mix, 2×, 10 μL) was prepared using GoTaq®qPCR Master Mix.

The PCR conditions were as follows: 5 min at 95°C, followed by 40 cycles of 15 s at 95°C, 1 min at 60°C. Relative gene expression was determined by the $2^{-\Delta\Delta Ct}$ method (where Ct = threshold cycle) using β-actin as a reference gene. Quantitative real-time PCR primer sequences were shown in Tab. 1.

Western blot analysis

Proteins from cells subjected to every treatment were extracted in a lysis buffer. After lysis on ice for 30 min, cell lysates were then clarified by centrifugation at 12,000 rpm at 4°C for 10 min. Protein concentrations were determined by BCA assay. Immunoblot analysis of protein expressions of LTA4H and GAPDH was performed as described in our previous report. Briefly, 30 μg of protein extracts were separated by 12% SDS–polyacrylamide gels; then, proteins were transferred onto PVDF membranes that were purchased from Millipore (Massachusetts, USA). The membranes were blocked with 5% nonfat milk powder in Tris-buffered saline/0.1% Tween-20 (TBST) for 2 h at room temperature and then incubated overnight with the primary antibody (LTA4H,1:500; GAPTH, 1:2000) at 4°C and anti-rabbit secondary antibody (1:5000) for 2 h at room temperature. After washing thrice, the immunoblots were detected by enhanced chemiluminescence (ECL) detection reagent. The relative band intensity of each protein was normalized for GAPDH.

TABLE 1

Primer sequences for RT-qPCR

Gene	Primer sequence (5'-3')
LTA4H	F:GGGGACCCTATCTTTGGGGA R:CAGGAGCATAGAGCCAGGCA
BLT1	F:CTTTGGTCCAGACCCCGAAG R:CGAAGATTCAGGAGGGGTGG
BLT2	F:GTCAATTGTGCCACCCATCG R:CTGTAGGAGATTGACCGCGT
GAPDH	F:TGCACCACCAACTGCTTAG R:GATGCAGGGATGATGTTC

Immunofluorescence analysis

FLS in 1 × 105 cells/mL were digested and then fully titrated into single cells, inoculated in 6-well plates for 24 h. After discarding the medium, cells were then incubated with 10 ng/mL TNF-α. The complete medium was added to the control group and was discarded after 24 h. The control and TNF-α group were then treated with 0.1% DMSO medium, and the drug groups were treated with different concentrations of Dioscin for 24 h.

After incubation in 0.5% H$_2$O$_2$ followed by normal goat serum to avoid nonspecific immunoreactions, the sections (N = 6, for each group) were incubated with primary antibodies of LTA4H at 4°C overnight. The next day, sections were washed with PBS for 15 min and incubated for 90 min at 37°C with fluorescent secondary antibody. Signals were measured using microscope image-analysis software (Image-Pro Plus, USA).

Statistical analysis

Values were expressed as $\bar{x} \pm s$, analyzed using one-way analysis of variance (ANOVA) and Student's t-tests in the program SPSS19.0 (USA). Nonparametric data (rate of swelling, histological analysis, and gene expression) was analyzed using the Mann–Whitney U-test. p-values of less than 0.05 were considered to be significant.

Results

Effects of TNF-α on FLS viability

MTT assay was done to calculate cell viability 24, 48, and 72 h after treating FLS with various concentrations (2.5, 5, 10, and 20 ng/mL) of TNF-α. As shown in Fig. 1, after treatment with TNF-α, FLS viability was increased significantly compared to the control group ($p < 0.05$). The highest cell survival and growth was observed in FLS exposed to 10 ng/mL TNF-α for 24 h. Thus, this culture condition of FLS was used for further experiments.

Effects of Dioscin on TNF-α induced FLS viability

MTT assay was done to assess the viability of TNF-α induced FLS at 24, 48, and 72 h, after treatment with or without various concentrations of Dioscin. As indicated in Fig. 2, the cell viability of the TNF-α model group was highest. Dioscin at the concentrations of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 μg/mL markedly inhibited the survival and growth of FLS induced by TNF-α in a time/dose-dependent manner ($p < 0.05$), except the 0.5 μg/mL Dioscin group which showed no inhibition on cell viability. The cell viability of TNF-α induced-FLS was 49.46 ± 1.33 after being treated with 3 μg/mL Dioscin for 24 h, which was the closest to 50% cell viability. Based on these results, we selected TNF-α-induced FLS cultured with 1, 2, 3,

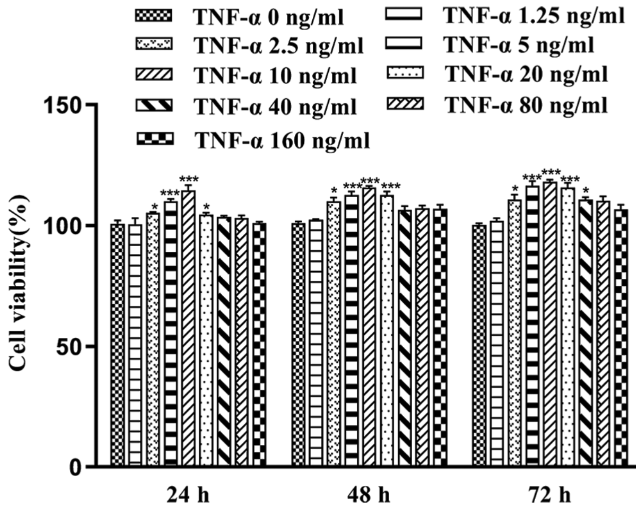


FIGURE 1. Cell viability of FLS at different incubation times with different concentrations of TNF-α ($\bar{x} \pm s$, N = 6), *$p < 0.05$, ***$p < 0.01$ *vs.* control group.

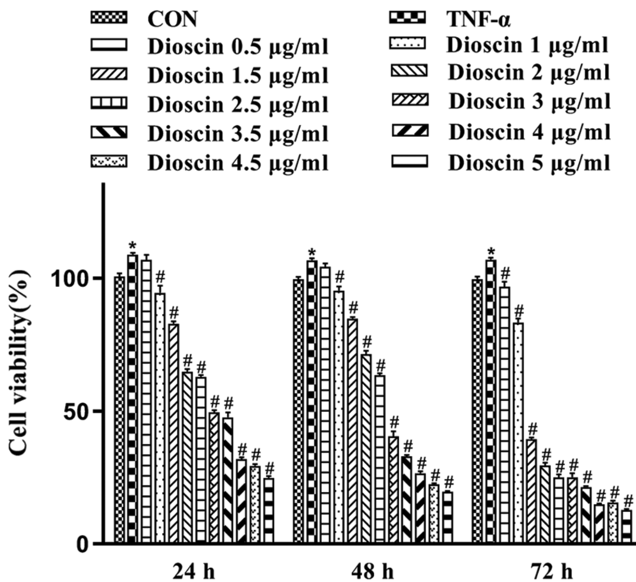


FIGURE 2. Cell viability of FLS after treatment with different concentrations of Dioscin for different times ($\bar{x} \pm s$, N = 6).
*$p < 0.01$ *vs.* control, #$p < 0.05$ *vs.* TNF-α group.

and 4 μg/mL Dioscin for 24 h to conduct further experiments (Fig. 2).

Effect of Dioscin on the LTB4 concentration in supernatant of TNF-α-induced FLS culture

As shown in Fig. 3, after treatment with 10 ng/mL TNF-α for 24 h, the amount of LTB4 in the cell culture medium significantly increased when compared with the control group ($p < 0.05$). The TNF-α-induced high LTB4 content was significantly decreased after Dioscin treatment in a dose-dependent manner ($p < 0.05$).

Effect of Dioscin on the expression of LTA4H in TNF-α-induced FLS

Compared with the control group, the expression levels of LTA4H mRNA and proteins in the TNF-α model group were significantly increased ($p < 0.05$); treatment with

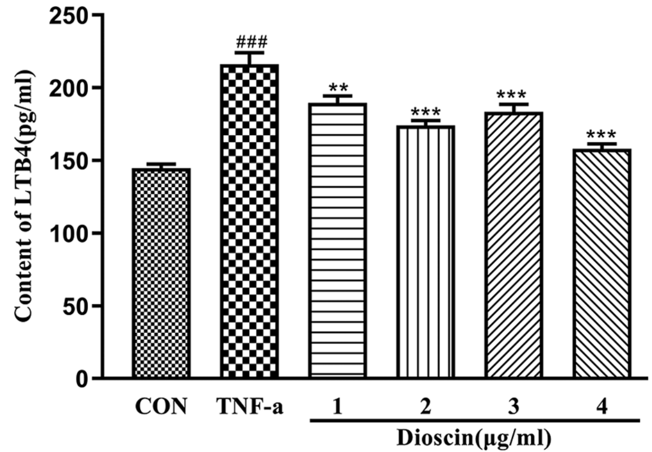


FIGURE 3. Effect of Dioscin on LTB4 content in TNF-α induced FLS supernatant ($\bar{x} \pm$ s, N = 10).
###$p < 0.001$ *vs.* control, **$p < 0.01$ *vs.* TNF-α group, ***$p < 0.001$ *vs.* TNF-α group.

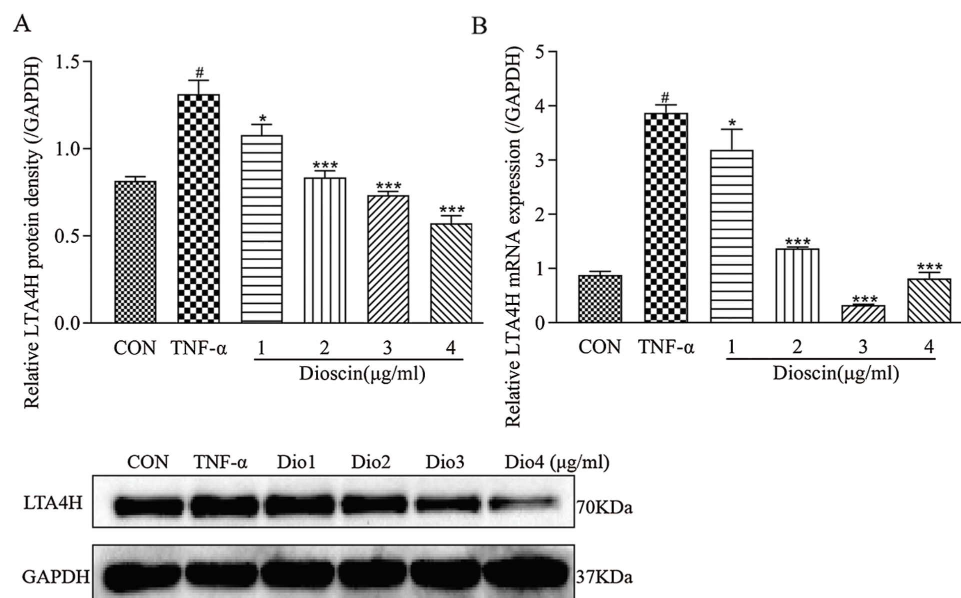
different concentrations of Dioscin significantly reduced the level of LTA4H mRNA and protein expression in TNF-α induced FLS ($p < 0.05$). At the 3 μg/mL Dioscin concentration, the expression level of LTA4H mRNA was the lowest, while treatment with Dioscin at different concentrations exhibited the inhibitory action in a dose-dependent manner and decreased the expression level of LTA4H protein induced by TNF-α in FLS (Fig. 4).

Effect of Dioscin on the expressions of LTB4 receptor in TNF-α-induced FLS

Compared with the control group, the mRNA expressions of BLT1 and BLT2 in the TNF-α group increased markedly, of which the expression level of BLT1 mRNA was six times, and BLT2 was three times higher than those in the control group. Dioscin treatment at 1, 2, and 3 μg/mL reduced the expression levels of BLT1 and BLT2 mRNA at different degrees; however, there were no significant differences between these groups. Treatment with 4 μg/mL Dioscin did not significantly change BLT1 mRNA expression but decreased the expression of BLT2 mRNA significantly compared with the other three groups (Fig. 5).

Discussion

RA is characterized by inflammatory cell infiltration, synovial tissue hyperplasia, bone erosion, and even joint deformity, leading to joint pain and disability, which severely breaks the life quality of RA patients (Wu *et al.*, 2014; Li *et al.*, 2020). FLS plays an important role in the pathogenesis of RA due to its inducing synovial hyperplasia. FLS can also enhance leukocytes' adhesion and migration into the endothelium and produce high concentrations of CXCL12 to inhibit leukocyte outflow from the joint in RA patients (McGettrick *et al.*, 2009; McGettrick *et al.*, 2010). In the synovium of RA, FLS loses the function of contact-inhibition but exhibits characteristics of tumor-like proliferation, such as proliferation acceleration, apoptosis reduction, and slowing cell aging (Pang *et al.*, 2016). Zou *et al.* (2017) discovered that FLS is an ideal target to



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CON TNF-α 1 2 3 4μg/ml

FIGURE 4. Effect of Dioscin on LTA4H expression in TNF-α induced FLS.
(A) The change of LTA4H protein expression was evaluated by western blotting. (B) The effect of Dioscin on LTA4H mRNA was assessed by quantitative real time PCR. (C) Effect of Dioscin on LTA4H protein expression was determined by immunofluorescence. Scar bar: 50 μm. ($\bar{x} \pm s$, N = 3). #$p < 0.001$ *vs.* control, *$p < 0.05$ *vs.* TNF-α group, ***$p < 0.05$ *vs.* TNF-α group.

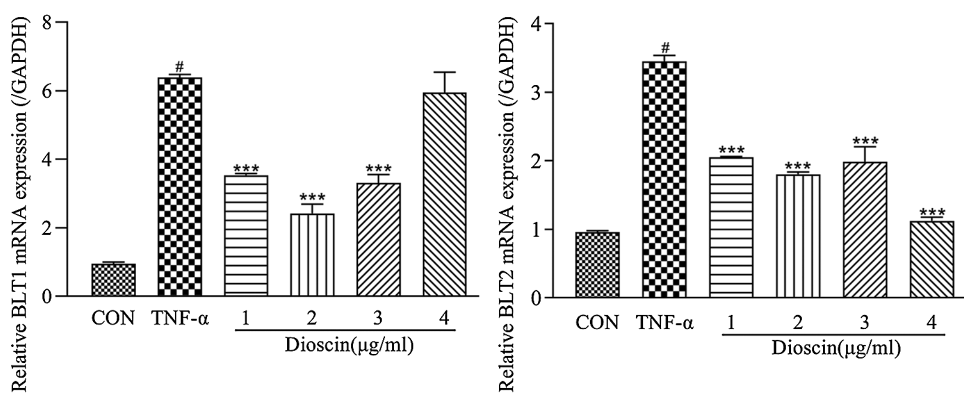

FIGURE 5. Effect of Dioscin on mRNA expressions of LTB4 receptors BLT1 and BLT2 in TNF-α induced FLS ($\bar{x} \pm s$, N = 3).
#$p < 0.001$ *vs.* control, ***$p < 0.05$ *vs.* TNF-α group.

investigate a drug's therapeutic effects of RA. Previous studies have indicated that the abnormalities of FLS proliferation/apoptosis may be related to inflammatory mediators for the treatment of RA (Crowley *et al.*, 2017; Huang *et al.*, 2017; Sun *et al.*, 2017). TNF-α can induce an excessive activation and proliferation of FLS to produce various kinds of inflammatory factors, leading to synovial hyperplasia and joint damage in RA disease (Pap *et al.*, 2000; Yamanishi and Firestein, 2001). Moreover, exogenous TNF-α has been proved to induce expression and release of endogenous TNF-α, which causes inflammation reactions *in vitro*, suggesting that the *in vitro* RA model could be constructed by TNF-α treated FLS (Zhang and Xiao, 2017). In fact, TNF-α induced FLS has been used as an *in vitro* cellular model of RA to investigate RA pathogenesis and its underlying pharmacological mechanisms (Tian *et al.*, 2010; Zhang and Xiao, 2017). In the present study, 10 ng/mL TNF-α for 24 hFLS proliferation was significantly increased after co-incubating with 10 ng/mL TNF-α for 24 h. The above result is consistent with previous studies (Tian *et al.*, 2010).

As an important steroidal saponin, Dioscin becomes a crucial raw material in steroid hormone drug synthesis (Wang *et al.*, 2017). Although Dioscin has been reported in CIA animal treatments, its underlying mechanisms for RA

treatment are still not fully clear (Gao *et al*., 2012; Guo *et al*., 2015; Lu, 2017). Using an *in vitro* RA model, the MTT assay was employed to investigate the appropriate functional concentrations of Dioscin. Our results showed that Dioscin's concentrations of higher than 1 μg/mL inhibited the proliferation of FLS induced by 10 ng/mL TNF-α treatment for 24 h, in which the inhibition ratio of cell proliferation was close to 50% after treating FLS using 3 μg/mL Dioscin for 24 h, indicating that Dioscin may promote FLS apoptosis in a time/dose-dependent manner. These findings suggest the potential therapeutic function of Dioscin against RA.

Higher LTB4 concentration was observed in the synovia and serum of RA patients (Elmgreen *et al*., 1987). LTB4 can activate and accumulate many inflammatory factors and immune cells, induce joint swelling and pain, as well as bone destruction, and is directly involved in the occurrence and development of arthritis (Tak and Bresnihan, 2000). Xu *et al*. (2010) found that the mRNA and protein levels of IL-1β and TNF-α were significantly increased when exogenous LTB4 was added or endogenous LTB4 was produced by lipopolysaccharide stimulation in primary FLS of RA patients, suggesting the involvement of LTB4 in RA pathogenesis. Bi *et al*. (2017) have also shown that LTB4 exhibited significant toxic effects on synovial cells and promoted apoptosis. Above all, LTB4 plays an important role in the development and progression of RA, and thus, it can be a crucial target for RA therapy. However, up till now, whether Dioscin's treating RA is related to its effect on LTB4 remains to be investigated. Our results showed, for the first time, that Dioscin inhibited the increase of LTB4 in TNF-α-induced FLS in a dose-dependent manner, indicating that one of the protective effects of Dioscin against RA may be achieved by reducing LTB4 generation. In addition, considering LTA4H is responsible for LTA4 transformation into LTB4, which is a direct functioning enzyme to produce LTB4, we further detected the influence of Dioscin on LTA4H. Our results showed that Dioscin decreased the mRNA and protein expressions of LTA4H in TNF-α-induced FLS at different degrees, firstly suggesting that Dioscin reducing LTB4 generation against RA may be due to its down-regulating LTA4H expression in TNF-α-induced FLS.

It is well known that LTB4 plays a critical role through the two receptors BLT1 and BLT2, among which the affinity of LTB4 with BLT1 is higher than that with BLT2, and it is easy to determine and obtain BLT1, so LTB4 functions via the BLT1 pathway during the treatment of RA and other inflammatory diseases (Yokomizo *et al*., 2000; Yokomizo, 2015). Chen *et al*. (2010) have found that FLS produced enough LTB4 to promote arthritis by cross-cell metabolism, and LTB4 levels were increased by 3-fold after TNF-α stimulation of FLS; furthermore, by activating BLT1, high levels of LTB4 regulated the migration and invasion of FLS to promote joint erosion and participate in the occurrence as well as the persistence of RA. For mediating the therapeutic effects against RA, LTB4 functions through the BLT2 pathway, which will be studied later. Mathis *et al*. (2010) found that arthritis and bone erosion of BLT2-knockout mice were significantly reduced, during which BLT1 expression did not change, indicating the important role of BLT2 in the incidence of RA. At present, there are no reports on the relationships between Dioscin treating RA and LTB4 receptors. Our results, for the first time, discovered that after TNF-α stimulation, the mRNA expression levels of BLT1 and BLT2 of FLS were significantly increased, and Dioscin reversed these alterations in TNF-α-induced FLS. These findings indicate that Dioscin can downregulate the expressions of BLT1 and BLT2, which may block the downstream pathway of LTB4 action and exert its therapeutic effect on TNF-α-induced FLS from CIA rats.

In conclusion, our primary experiments firstly demonstrated that Dioscin produces a protective effect on TNF-α-induced FLS injury via its decreasing LTB4 production by down-regulating the expression of LTA4H, and it may also involve its inhibiting LTB4 downstream pathway by down-regulating the expressions of BLT1 and BLT2. These findings firstly suggest that Dioscin produces a potential therapeutic effect for RA via its influencing LTA4H/LTB4/BLT pathway.

Acknowledgement: Not applicable.

Availability of Data and Materials: The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Author Contribution: Zhiping Wei contributed to acquisition of data; Yajun Liu, Meiwen Yang, MengdiLi, Kexin Li contributed to analysis and interpretation of data; Luxi Zheng, Huiqiong Guo contributed to paper revision in grammars and languages; Fenfang Hong and Shulong Yang are responsible for study conception and design and fund. analysis and interpretation of results, and manuscript preparation.

Ethics Approval: The study was approved by the Ethics Committee of College of Medicine in Nanchang University.

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Conflicts of Interest: The authors declare that there are no conflicts of interests to report regarding the present study.

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