Dihydroarteannuin Ameliorates Collagen-Induced Arthritis Via Inhibiting B Cell Activation by Activating the FcγRIIb/Lyn/SHP-1 Pathway

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Background: Dihydroarteannuin (DHA), which is extracted from the traditional Chinese herb Artemisia annua L., exhibits potent immunosuppressive activity in rheumatoid arthritis (RA). Strong evidence indicates that B cells act as an essential factor in the pathogenesis of RA, but research on the immunosuppressive function of DHA in regulating B cells is limited.

Objective: To investigate the modulatory effects of DHA on joint destruction, proinflammatory cytokine production, activation, apoptosis and proliferation of B cells and to explore the possible associated mechanism in RA treatment.

Methods: Collagen-induced arthritis (CIA) model was established. Weight and joint oedema were record weekly, and joint damage was detected by micro-CT scan. Human Burkitt B lymphoma cells lacking endogenous Fc gamma receptor b (FcγRIIb) gene were transfected with a 232Thr loss-of-function mutant to construct a mutant cell model ST486. The proliferation of ST486 cells was assessed with Cell Counting Kit-8. Apoptosis and activation were tested by flow cytometry. The effects of DHA on the activation of FcγRIIb, protein tyrosine kinases (Lyn), and SH2-containing tyrosine phosphatase-1 (SHP-1) signaling pathways were determined by western blotting.

Results: In comparison to model group, bone volume/tissue volume (BV/TV) and bone mineral density (BMD) were increased, whereas joint oedema was decreased in both of the DHA and MTX group. The mRNA and protein expression levels of Interleukin-6 (IL-6) and Tumor necrosis factor-alpha (TNF-α) were decreased after treatment with DHA. Moreover, the expression of phosphorylated CD19 was also inhibited by DHA.

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**HIGHLIGHTS**

- Studies on the immunosuppressive function of DHA in regulating B cells are limited.
- Restoring the inhibitory function of FcγRIIB may be a new strategy for the treatment of RA.
- We used gene transfection technique to construct FcγRIIB mutant cell line ST486.
- DHA inhibits B cell activation by activating the FcγRIIB/Lyn/SHP-1 pathway.
- This is the first report showing that DHA achieves these effects by activating this pathway.

**INTRODUCTION**

Rheumatoid arthritis (RA) is a common autoimmune disease, which seriously endangers human health. Although the etiology is still unknown, many studies have shown that genetic defects and environmental factors interact to cause the pathogenesis of RA (Smolen et al., 2016). Genetic, immunological and clinical studies show that B cells play a key role in the occurrence, development and treatment of RA. Activated B cells produce a large number of antibodies against autoantigens, which is one of the main differences between RA and other inflammatory arthritis, including rheumatoid factors (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies, which are present in patients’ sera before the clinical symptoms of RA (van Gaalen et al., 2005). Additionally, a randomized clinical study have shown that Rituximab, a monoclonal antibody against B cell, not only significantly improves the autoantibody level of RA patients, but also reduces radiological progress. Its efficacy is closely related to the clearance of memory B cells, strongly suggesting the importance of B cells in RA (Nakou et al., 2009; Chatzidionysiou et al., 2016).

Fc gamma receptor b (FcγRIIb) is an important molecule in immune regulation, which can prevent excessive activation of B cell receptor (BCR) signals and reduce the risk of autoimmune diseases. The down-regulation or functional deficiency of FcγRIIb will not only increase the incidence of RA or systemic lupus erythematosus (SLE), but also aggravate the joint destruction of RA (Nimmerjahn and Ravetch, 2006). In addition, a study of single nucleotide polymorphism (SNP) in 246 patients with RA was followed up for 6 years and it was found that the 695T>C (Ile232Thr) polymorphism in exon five of FcγRIIb gene could attenuate the signal of inhibitory receptor and aggravate the condition of RA and joint destruction (Li et al., 2003; Radstake et al., 2006). More importantly, a clinical study showing that FcγRIIb can be regulated, it is dysfunctional in active RA but normal in inactive RA where the disease is controlled (Magnusson et al., 2014). Additionally, after FcγRIIb binds to the BCR and tyrosine phosphorylates, it can bind to SH2-containing tyrosine phosphatase-1 (SHP-1). The effect of SHP-1 on the function of B cells is not only on their proliferative capacity, but also on apoptosis and cell killing, which can be regulated by interacting with different molecules downstream of B cell surface receptors (Raghav et al., 2018a; Raghav et al., 2018b; Raghav et al., 2018c).

Disease-modifying antirheumatic drugs (DMARDs) are the basic drugs for the treatment of RA (Singh et al., 2015), especially methotrexate (MTX) is the anchoring drug of RA. In spite of this, these drugs still have many side effects (Otero et al., 2017). Therefore, the development of alternative drugs with fewer side effects is very important to achieve a better clinical outcome (Zhang et al., 2010).

Dihydroartemisinin (DHA; MW: 284.35, molecular formula: C_{15}H_{24}O_{3}), a semisynthetic derivative of artemisinin, is a widely used antimalarial drug with anti-inflammatory, anticancer, and immunosuppressive activities (Yu et al., 2021). Increasing evidence suggests that DHA exerts an immunosuppressive function in several autoimmune diseases, including RA and SLE (Li et al., 2006; Fan et al., 2018a; Li et al., 2019; Wang et al., 2021). Previous studies have found that DHA promotes apoptosis in B cells and that B cell lymphoma-2 (Bcl-2) is an anti-apoptotic protein that determines B cell apoptosis by interacting with pro-apoptotic members of the Bcl-2 family (Raghav et al., 2012a; Raghav et al., 2012b; Raghav et al., 2019). Therefore, we speculate that DHA may promote B-cell apoptosis by inhibiting Bcl-2 to achieve therapeutic effects in RA. In addition, some studies have demonstrated that DHA can diminish CD8+ T cell memory, affect Th and regulatory T cell functions, restore the Treg/Th17 cell balance, and so on (Zhao et al., 2012; Fan et al., 2018b; Chen et al., 2020). However, how DHA affects the function of B cells is still unclear. More importantly, the potential immune regulation mechanism of DHA is worthy of further study.

In the present study, CIA in DBA/1 mice was established. Human Burkitt B lymphoma cells (lacking endogenous FcγRIIb gene) were transfected with a 232Thr loss-of-function mutant to construct a mutant cell model ST486. We investigated the effects of DHA on joint damage in CIA mice, and the proinflammatory cytokine production, activation, proliferation and apoptosis of ST486 cells. In addition, we further confirmed whether these effects correlated with the FcγRIIb/Lyn/SHP-1 signaling pathway.

**Conclusion:** We provide the first evidence that DHA may alleviate collagen-induced arthritis by activating the FcγRIIb/Lyn/SHP-1 signaling pathway in B cell, indicating that DHA is a novel and valuable candidate for RA therapy.

**Keywords:** dihydroartemisinin, FcγRIIb, rheumatoid arthritis, ST486, CIA mice
MATERIALS AND METHODS

Chemicals and Reagents
DHA (D7439, 150 mg), lipopolysaccharide (LPS), phorbol myristate acetate (PMA) and MTX hydrate (M8407) were purchased from Sigma-Aldrich (St. Louis, MO, United States). Complete Freund's adjuvant (CFA) (7001, 10 ml), incomplete Freund's adjuvant (IFA) (7002, 10 ml), and bovine type II collagen (20021, 10 ml) were purchased from Chondrex, Inc. (Redmond, WA, United States). ST486 cell line (70014502, CRL-1647), RPMI 1640 culture medium and fetal bovine serum (FBS) were purchased from ATCC Co., Ltd. (United States). AffiniPure goat anti-human IgM (109-005-129) was obtained from Jackson ImmunoResearch Co., Ltd. (PA, United States). Flu-4 AM (F312) was purchased from Dojindo Co., Ltd. (Japan). The primary antibody against CD32B (ab45143, EP888Y) was purchased from Abcam Co., Ltd. (Cambridge, United Kingdom). The primary antibody against GAPDH (10494-1-AP) was obtained from Proteintech Group, Inc. (United States). The primary antibodies against Lyn (C13F9) (2796), SHP-1 (C14H6) (3759), and phospho-C1219 (Tyr531) (3571) were purchased from CST Co., Ltd. (United States).

Animals
The animals used for this experiment were male DBA/1J mice (Vital River Laboratory Animal Technologies Co. Ltd. Beijing, China) that were 8 weeks old. The mice were housed in a laminar flow cabinet with a 12 h light/dark cycle and maintained on specific pathogen-free (SPF) laboratory chow and water ad libitum. All the experimental studies were strictly in accordance with Guangzhou University of Chinese Medicine Animal Ethics Committee guidelines for the rational use of animals.

Induction and Evaluation of Collagen-Induced Arthritis
After 1 week of adaptation, 24 DBA/1J mice were randomly divided into two groups: control group (n = 6), arthritis-induced group (n = 18). To prepare mouse CIA model, 0.1 ml of emulsion of bovine type II collagen and CFA (1:1, v/v) was injected intradermally into the tail base of mice in arthritis-induced group on day 0, as the primary immunization. A booster injection of 0.1 ml of emulsion of bovine type II collagen and IFA (1:1, v/v) was administered intradermally into the back on day 21, as the secondary immunization (Zhang et al., 2013; Miyoshi and Liu, 2018). Mice in control group injected normal saline at the same location and frequency as arthritis-induced group. The clinical symptoms were recorded weekly since the secondary immunization, with the arthritis index scores according to the following criteria: 0 = normal; 1 = erythema and mild swelling; 2 = erythema and swelling extending to ankle joints and one or two toes; 3 = erythema and swelling extending to metatarsal joints and more than two toes; and 4 = ankylosing deformity with joint swelling. The scores from each paw were added to obtain a cumulative score between 0 and 16. In addition, swelling in the paw was measured using digital calipers, and body weight was recorded during the course.

Drug Administration
After the secondary immunization on day 21, mice in the arthritis-induced group were randomly divided into three groups (n = 6 per group): model group, DHA group (20 mg/kg, daily), MTX group (2 mg/kg, every 3 days). DHA and MTX was dissolved in corn oil and administrated by oral gavage from day 21 to day 49. Control and model mice were orally given an equal volume of corn oil in parallel.

Micro-CT Analysis
After 4 weeks of drug administration, the right hind knee and ankle of each mouse was withdrawn and scanned by Skyscan 1176 micro-CT scanner (Bruker micro-CT, Kontich, Belgium). The scanning was carried out using following settings: voltage, 80 kV; source current, 88 μA; pixel size 4 μm. Two and three-dimensional images were generated using Data-viewer and CTvol softwares (Bruker micro-CT, Kontich, Belgium) respectively. The bone mineral density (BMD) and bone volume/tissue volume (BV/TV, %) were measured using CT Analyser program (Bruker micro-CT, Kontich, Belgium).

Plasmid Constructs, Transfections, and ST486 Cell Culture
ST486 cells were maintained in RPMI-1640 medium in a humidified chamber with 37°C at 5% CO₂. ST486 cells that expressed wild-type human FcRIIb or the I232T loss-of-function mutant were constructed. Murine stem cell virus (pMSCV) puro plasmids contained human FcRIIb-I232T or FcRIIb-WT were used as templates, and inserted into lentiviral expression vector (pLVX) via the EcoRI and MluI restriction enzyme sites after PCR amplification. Then the expression vector constructs of human FcRIIb-WT or FcRIIb-I232T were transfected to ST486 by electroporation. The stable sublines of ST486 cells expressing equivalent levels of human FcRIIb-I232T or FcRIIb-WT were sorted by at least two rounds of fluorescence-activated cell sorting. Quantitative real-time PCR (qRT-PCR) assays were performed to verify the transfection. The methods for RNA purification and qRT-PCR will be described below. The specific primer sets in Supplementary Table S1. After the transfection, transfected cells (Mut group) were incubated with or without different concentrations of DHA or MTX to perform the following studies.

Measurement of Proinflammatory Cytokine Levels by Enzyme-Linked Immuno Sorbent Assay
ELISA kit (Beijing 4A Biotech Co., Ltd., Beijing, China) were used to determine the cytokine production under DHA
treatment. Transfected cells (Mut group) were divided into six groups: NC (without DHA and LPS), LPS (150 ng/ml), DHA (250 ng/ml), DHA (500 ng/ml), DHA (1000 ng/ml), and MTX (500 ng/ml, positive control group). Twenty-four hours after transfection, the cells (1 × 10^5/ml) were seeded in 24-well plates and treated with different concentrations of DHA or MTX (500 ng/ml) for 48 h, then PMA was added and incubated at 37°C with 5% CO2 for 48 h. The supernatant was then discarded, LPS (150 ng/ml) was added to each group except the NC group, and the cells were incubated overnight at 37°C with 5% CO2. The culture supernatants were collected, and the amount of TNF-α released from the cells was detected according to the manufacturer’s instructions. IL-6 cytokine assays were carried out using the same method.

**RNA Purification and a Quantitative Real-Time PCR Assay**

qRT-PCR was performed to analyze the expression of TNF-α and IL-6 in ST486 cells treated with DHA or MTX. Total RNA was isolated with TRIzol (Invitrogen, United States) and reverse transcribed into cDNA using a Prime Script RT Reagent kit (Takara Biotechnology, Dalian, China) according to the manufacturer’s protocol. qRT-PCR was completed after 40 cycles of 95°C for 30 s, 60°C for 1 min, and 72°C for 1 min. The relative fold-changes of gene transcriptions were determined by 2^-△△CT_ method with the specific primer sets in Supplementary Table S1.

**Cell Viability Assay**

The experiment was divided into six groups, which involved FcyRIIb-WT (control group), FcyRIIb-Mut (model group), MTX (500 ng/ml, positive control group), DHA (250 ng/ml), DHA (500 ng/ml) and DHA (1000 ng/ml). 10 μL of CCK-8 solution was added after culturing with different concentrations of DHA or MTX for 48 h. Then cells were incubated under the same conditions for another 2 h. The absorbance at 490 nm was measured by a microplate reader (iMark, Thermo Fisher Scientific, United States) to evaluate cell viability.

**Apoptosis Assays**

Annexin V apoptosis detection kit (BD Biosciences, United States) was used to measure cell apoptosis. After treating with different concentration of DHA or MTX as described above, the transfected cells were harvested and incubated with 7-AAD and Annexin V antibody following the manufacturer’s instructions. Then, cells were immediately analyzed by a FACSCalibur flow cytometer.

**Measurement of Cytoplasmic Ca^{2+} Concentrations**

Multiple signal transduction pathways can affect changes in intracellular Ca^{2+} concentration, and thus the detection of intracellular Ca^{2+} changes can help to understand the initiation, enhancement or inhibition of cellular functions. In this study, we used flow cytometry to monitor the dynamic changes of intracellular Ca^{2+} concentration in order to dynamically observe the degree of B-cell activation and thus verify whether DHA can inhibit the B-cell activation. After treating with different concentration of DHA or MTX as described above, the transfected cells (1.5 × 10^5/ml) were incubated in RPMI 1640 medium containing Fluo-4 AM (5 μM) for 1 h in a humidified chamber with 37°C at 5% CO2 to load with Fluo-4 AM. Then it was washed 3 times and detected by a flow cytometer constantly. The cells were stimulated with anti-human IgM (20 μg/ml) at 50 s, CaCl2 was added back to a final concentration of 2 mM at 400 s, and intracellular Ca^{2+} flux was measured for 800 s.

**Western Blot Analysis**

After treating with different concentration of DHA or MTX as described above, cells were stimulated with anti-human IgM (20 μg/ml) for 15 min. Protein lysates obtained from equal numbers of ST486 cells were separated by SDS-PAGE and then transferred to 0.4 μm PVDF membranes via electroblootting. After being blocked by skimmed milk for 1 h, membranes were incubated with antibodies against GAPDH, phospho-CD19, CD32B, Lyn, and SHP-1 overnight at 4°C. Then the membranes were incubated with the corresponding secondary antibodies at room temperature for 1 h. GAPDH was used as an internal control. Immunoreactive bands were visualized through Enhanced Chemiluminescence method and recorded in gel documentation system.

**Statistical Analysis**

All experiments were repeated at least three times, and all data are presented as the mean ± SEM. Statistics were analyzed using SPSS (version 20.0, IBM, Armonk, NY) while graphs were drawn by GraphPad Prism software (version 7.0). Significant differences between multiple groups were calculated using one-way ANOVA followed by Tukey’s test. Values of p < 0.05 were considered significant.

**RESULTS**

**Dihydroarteannuin Alleviated Joint Destruction in Collagen-Induced Arthritis Mice**

DHA and MTX treatment did not cause weight loss in CIA mice (Figure 1A). Mice in DHA (20 mg/kg, daily), MTX (2 mg/kg, every 3 days) group showed significant reduction in paw oedema between day 28 and day 49 compared with mice in model group (p < 0.05, Figures 1B,C). Collagen challenge induced arthritis in mice, as evidenced by red swelling in the paw (Figure 1D). Micro-CT analysis showed that DHA or MTX treatment ameliorated the bone loss, as BMD and BV/TV in both DHA and MTX group were significantly higher than those in model group (p < 0.05,
FIGURE 1 | DHA alleviated inflammation and joint destruction in CIA mice. (A) Body weight were recorded weekly during the experiment. (B) The thickness of hind paws was measured weekly by digital calipers. (C) Arthritis index score was used to score each mice every week, with the highest score of 16. (D) Images of swollen hind paws of mice. The right hind ankle and knee of mice were scanned by the Skyscan 1176 Micro-CT Imaging System. (E-H) The BV/TV and BMD in knee and ankle were calculated. *p < 0.05, **p < 0.01 vs. the model group. "p < 0.05, ""p < 0.01 vs. the control group.
Figures 1D–H). Besides, no significant difference was observed between DHA and MTX groups in any of the above analyses.

**FcγRIIb mRNA Expression in Transfected ST486 Cells**

As seen in **Figure 2A**, after the plasmid was digested, an obvious band could be seen around 8000bp, and there were 23bp bases between the two digestion sites, so it could not be detected. After the plasmids were digested by EcoRI + MluI, obvious bands could be seen at 8000 bp and 1000 bp attachment, indicating that the 951 bp sequence of the target gene had been inserted. The sequencing results showed correct and the plasmids were confirmed. **Figure 2B** shows the sequence maps of wild-type and variant FcγRIIb encoding membrane penetrating proteins, showing that variant FcγRIIb has a variation at site 232. As shown in **Figure 2C**, successful plasmid transfection was verified by qRT-PCR, and our results showed that when compared to the WT group, the relative expression of FcγRIIb was significantly decreased in both of the Con and Mut groups ($p < 0.0001$ and $p < 0.01$, respectively).

**Dihydroartemisinin Suppresses Proinflammatory Cytokine Expression Stimulated by Lipopolysaccharide**

Accumulated evidence reveals that certain proinflammatory cytokines contribute to the pathogenic factors supporting the proliferation and activation of B cells (Carvalho et al., 2011; Gottenberg et al., 2012; Lee et al., 2017). Therefore, we assessed the role of DHA in the regulation of inflammatory cytokine production by LPS-stimulated ST486 cells. As shown
in Figures 3A,B, compared with the LPS group, the protein expression levels of TNF-α and IL-6 were significantly lower in both the DHA and MTX-treated groups, except for the protein expression level of TNF-α in the DHA (250 ng/ml) group. In addition, as seen in Figures 3C,D, we also found that IL-6 and TNF-α mRNA levels were decreased in the DHA-treated group compared to the LPS group, while the difference compared with the MTX group was not statistically significant.

The Proliferation of ST486 Cells Were Suppressed Under Dihydroarteannuin Treatment

CCK-8 is used to detect the proliferation of ST486 cells and to evaluate the influence of DHA on the proliferation of ST486 cells. Figure 4A showed that when compared with those in the Mut group, the proliferation of ST486 in the WT, MTX and different dose of DHA group were all significantly decreased (p < 0.0001, p < 0.0001, p < 0.0001, and p < 0.0001, respectively). Besides, when compared with the MTX group, the proliferation of ST486 in the different dose of DHA group were all significantly increased (p < 0.01, p < 0.05, and p < 0.05, respectively).

Dihydroarteannuin Promoted the Apoptosis of ST486 Cells

The apoptotic cells were detected by flow cytometry. As shown in Figures 4B,C, when compared with that in the Mut group, the number of apoptotic cells in the WT, MTX and DHA-treated groups were all significantly increased (p < 0.0001, p < 0.0001, p < 0.0001, and p < 0.0001, respectively). Additionally, when compared with the MTX group, the numbers of apoptotic cells in the DHA-treated (250 ng/ml) was significantly decreased (p < 0.0001), the numbers of apoptotic cells in the WT group was increased (p < 0.0001).

Dihydroarteannuin Regulated the CD19 Pathway and Decreased Intracellular Ca2+ Flux in ST486 Cells

Previous studies have showed that FcγRIIb selectively dephosphorylated CD19 in consequence with an inhibition

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**FIGURE 3** | DHA inhibited the protein and mRNA expression of inflammatory cytokines in ST486. **(A,B)** The protein expression level of TNF-α and IL-6 were detected by ELISA. **(C,D)** The mRNA expression level of TNF-α and IL-6 were detected by qRT-PCR. The experiments were repeated three times and analyzed using one-way ANOVA. “p < 0.05,” “p < 0.01,” “p < 0.001” and “p < 0.0001” vs. the LPS group; “p < 0.05” and “p < 0.0001” vs. the MTX group.
of BCR signaling (Koncz et al., 1998). Therefore, the western blot analysis was used to investigate whether the effect of DHA is correlated with the CD19 pathway. When compared to the cells in the WT group, the intracellular Ca²⁺ level in the Mut group was remarkably increased after 100 s. However, when compared with the Mut group, the calcium concentration decreased more rapidly in DHA and MTX group after 200 s (Figure 5B). Besides, as illustrated in Figure 5F, when compared with the Mut group, the protein expression levels of phosphorylated CD19 were all downregulated in the WT, DHA-treated (500 and 1000 ng/ml) and MTX groups (p < 0.05, p < 0.05, p < 0.05, and p < 0.01, respectively). These data all indicated that DHA inhibited B cell activation.

**Dihydroartemisinin Regulated the FcγRIIb/Lyn/SHP-1 Pathway in ST486 Cells**

Previous studies have shown that FcγRIIb signal pathway plays an important role in B cell activation (Hippen et al., 1997), so we further study the effect of DHA on this signal pathway (Figure 5A). The results showed that when compared to those in the Mut group, there were a significant increased in CD32B, Lyn and SHP-1 expression in the WT group (p < 0.05, p < 0.05, p < 0.01, respectively).

![Figure 4](image-url)
and $p < 0.01$, respectively, Figures 5C–E). Additionally, the protein expression of CD32B in DHA (500 and 1000 ng/ml) groups were much higher than that in the Mut group ($p < 0.05$ and $p < 0.05$, respectively). Besides, the Lyn expression in the DHA (1000 ng/ml) and MTX groups were significantly higher than that in the Mut group ($p < 0.05$ and $p < 0.05$, respectively).

Correspondingly, when compared with the Mut group, consistent results for the SHP-1 expression in the DHA (1000 ng/ml) and MTX groups ($p < 0.01$ and $p < 0.05$, respectively). In all, these data suggested that DHA impeded B cells activation induced by IgM through the modulation of the FcγRIIb/Lyn/SHP-1 pathway.

**FIGURE 5** | Regulation of DHA on CD19/FcγRIIb-Lyn-SHP-1 pathway and effect of intracellular Ca$^{2+}$ flux in ST486 cells. (A) The cells were treated as above, and then stimulated with anti-human IgM (20 μg/ml) for 1 h. The protein expression levels of CD32b (FcγRIIb), GAPDH, Lyn, phospho-CD19 and SHP-1 were detected by western blot. This part of the experiment was repeated three times. (B) The cells were incubated in RPMI 1640 medium containing 10% fetal bovine serum and Fluo-4 AM (5 μmol), incubated at 37°C for 1 h, washed 3 times, and continuously monitored by flow cytometry. Anti-human IgM (20 μg/ml) was added at 50 s, add CaCl$_2$ to the final concentration of 2 mM at 400 s, and the intracellular Ca$^{2+}$ flux was continuously detected to 800 s (C–F) With GAPDH as the internal reference, the relative expression of each protein was expressed as the protein/GAPDH. The experiments were repeated three times and analyzed using one-way ANOVA. *$p < 0.05$ and **$p < 0.01$ vs. the Mut group; *$p < 0.05$ vs. the Mut group.
DISCUSSION

To date, DMARDs have been the primary choice for RA patient treatment, especially MTX, which is the anchoring drug of RA (Singh et al., 2015). However, MTX is a slow-acting anti-rheumatic drug, its effect is slow. In addition, MTX also has a large number of side effects. Therefore, it is urgent to develop alternative drugs with low toxicity. DHA is the most active component of artemisinin, it is extracted from traditional Chinese herbal medicine Artemisia annua L. Previous studies have shown that DHA has immunosuppressive effects on some diseases, such as RA and SLE. However, the exact molecular mechanism of DHA in treating RA is still unclear. In our study, we demonstrated that DHA achieved therapeutic effects in joint destruction in CIA mice. Besides, DHA could not only suppress the proinflammatory cytokine production of B cells but also promote the apoptosis, and inhibit the proliferation and activation of these cells. More importantly, we provided the first evidence that DHA might achieve these effects by activating the FcYRIIb/Lyn/SHP-1 pathway.

Strong evidence indicates that B cell activation play a key role in the occurrence, development and treatment of RA (van Gaalen et al., 2005; Nakou et al., 2009; Chatzidionysiou et al., 2016). Previous studies have focused on autoantigen-driven B cell activation, but recent studies have found that B cells from RA patients have defects in autoimmune tolerance during bone marrow development. During the development of B cells, pre-B cells in bone marrow can greatly reduce the production of autoreactive B cells through the mechanisms of receptor rearrangement, clone deletion and Anergy, which is a normal central immune tolerance. But in spite of this, a small number (6%) of autoreactive B cells were released to the periphery (von Boehmer and Melchers, 2010; Pillai et al., 2011). At this time, the mechanism of peripheral B cell immune tolerance plays a role which is regulated by FcYRIIb. After BCR signal is activated by autoantigen, FcYRIIb inhibits CD19 phosphorylation by cross-linking with BCR, activates Lyn and immune receptor tyrosine inhibitory motif (ITIM), and recruits SHP-1. SHP-1 dephosphorylates multiple signal molecules, inhibits the metabolism of Ca²⁺, hinders the cascade of activation signals triggered by BCR, and induces immune tolerance (Karnell et al., 2014). However, the 695T > C (Ile232Thr) polymorphism in exon five of FcYRIIb gene could attenuate the signal of inhibitory receptor, aggravate the condition of RA and joint destruction. More importantly, a clinical study indicated that FcYRIIb can inhibit the activation of B cells. Overall, our data demonstrate that DHA achieves these effects through a process that associated with the FcYRIIb/Lyn/SHP-1 pathway. However, there are some shortcomings in our study. This study did not involve the validation of in vivo molecular mechanisms, other than the effects of DHA on patients were not assessed in our study. Therefore, further clinical studies will be conducted to evaluate the clinical efficacy of DHA.

In conclusion, as far as we know, our study first demonstrated that DHA can not only reduce the level of inflammation in CIA mice, but also reduce the risk of bone destruction. In addition, we found that DHA can not only inhibit the production of B cell pro-inflammatory cytokines, but also inhibit B cell proliferation and activation, and promote B cell apoptosis. More importantly, we have demonstrated for the first time that DHA can inhibit B cell activation by activating FcYRIIb/Lyn/SHP-1 signaling pathway, thus achieving the therapeutic effect of RA. Therefore, our study highlights the potential of DHA as an alternative drug for RA.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics Committee of Laboratory Animals in The First Affiliated Hospital of Guangzhou University of Chinese Medicine.

AUTHOR CONTRIBUTIONS

CH wrote manuscript. CH and DW. performed most experiments. GC designed and supervised the experiments as the PI. LL and MZ
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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.883835/full#supplementary-material
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GLOSSARY

ACPA anti-cyclic citrullinated peptide antibodies
BCR B cell receptor
BMD bone mineral density
BV/TV bone volume/tissue volume
Bcl-2 B cell lymphoma-2
CCP cyclic citrullinated peptide
CFA complete Freund’s adjuvant
CIA collagen-induced arthritis
DHA dihydroartemisinin
DMARDs disease-modifying antirheumatic drugs
ELISA enzyme-linked immuno sorbent assay
FBS fetal bovine serum
FcyRIIb Fc gamma receptor b
IFA incomplete Freund’s adjuvant
IL-6 interleukin-6
ITIM immunoreceptor tyrosine-based inhibitory motif
LPS lipopolysaccharide
Lyn protein tyrosine kinases
LVX lentiviral expression vector
MTX methotrexate
MSCV murine stem cell virus
PMA phorbol myristate acetate
qRT-PCR Quantitative real-time PCR
RA rheumatoid arthritis
RF rheumatoid factors
SHP-1 SH2-containing tyrosine phosphatase-1
SLE systemic lupus erythematosus
SNP single nucleotide polymorphism
SPF specific pathogen-free
ST486 Burkitt’s lymphoma ST486 human B cells
TNF-α tumor necrosis factor-alpha
WT wild type