Original Research

The effects of dihydroartemisinin on inflammatory bowel disease-related bone loss in a rat model

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Impact statement

Bone loss is one of the important extra-intestinal manifestations in patients with inflammatory bowel diseases (IBDs). Studies have shown that compounds derived from natural products are useful in the treatment of IBDs. However, few studies have investigated the role of compounds derived from natural products in treatment of osteoporosis in IBDs. The current study aimed to show the effects of dihydroartemisinin (DHA), antimalaria drug, on bone loss in a rat model of IBD. The findings showed that DHA intervention dose dependently protected against bone loss in IBD rats by inhibiting tumor necrotic factor α production and osteoclast formation. These findings highlights that DHA may be beneficial for bone health in those patients with IBD.

Abstract

Bone loss is one of the important extra-intestinal manifestations in patients with inflammatory bowel diseases (IBDs). Compounds derived from natural products have been used to treat IBDs. However, the role of natural products on IBD-induced bone loss is not completely clarified. In the present study, we observed the effects of dihydroartemisinin (DHA), an antimalaria drug, on IBD and IBD-induced bone loss in a rat model. Chronic IBD model was established in Sprague-Dawley rats by giving them 2.5% dextran sodium sulfate in drinking water. DHA was given by intraperitoneal injection. Blood, colon, and bone samples were collected for biomarker assay and histological analysis. There was an obvious increase in tumor necrotic factor (TNF) α and receptor activator of nuclear factor (NF)-kB ligand (RANKL), and decrease in procollagen type 1 N-terminal propeptide (P1NP) level in IBD groups compared with the normal control ($p < 0.05$). The disease activity score of IBD rats was significantly higher than the control ($p < 0.01$). Obvious decrease in disease activity score, TNFα, and RANKL level and increase in P1NP were observed in DHA-treated IBD rats. Bone loss, shown as the decrease in bone mineral density, bone volume fraction, and trabecular number and increase in trabecular separation were observed in IBD rats compared with control ($p < 0.01$). DHA treatment obviously abolished the bone loss, in particular in the high-dose group ($p < 0.05$). DHA treatment also inhibited the excessive osteoclast formation; RANKL protein expression; and *RANK*, *TRAF6*, *Fra-1*, *NFATc1* mRNA expression induced by IBD. Our data indicated that DHA may be a potential therapeutic agent for IBD and IBD-induced bone loss.

Keywords: Inflammatory bowel diseases, bone loss, tumor necrotic factor α, osteoclast, dihydroartemisinin, receptor activator of nuclear factor-kB ligand

Experimental Biology and Medicine **2018; 243: 715–724. DOI: 10.1177/1535370218769420**

Introduction

Inflammatory bowel diseases (IBDs) are common chronic gastrointestinal disorders characterized by inflammation and tissue degeneration in both children and adults.[1] The pathogenic mechanism of IBD is still unknown. Genetic, immune, and environmental factors may both play critical roles in IBD.[2] IBD patients suffer from chronic intestinal inflammation characterized by diarrhea, abdominal pain, and weight loss.[3] During the inflammatory process, the stimulated inflammatory cells, such as macrophages, dendritic cells, and antigen-presenting cells, secrete pro-inflammatory cytokines and chemokines, such as

interleukin (IL)-6, IL-12, and tumor necrosis factor (TNF),[4] which can cause systemic inflammation and extra-intestinal manifestations.[5–7]

Bone loss is one of the important extra-intestinal manifestations in IBD patients. Low bone mass was observed in 40–50% of IBD patients.[8] Many studies have shown that IBD patients have a high risk of osteoporosis and bone fractures.[7–9] Bone loss is related to a variety of factors, including weight loss,[7,10,11] systemic inflammation,[6,9,12] and treatment with corticosteroids.[9] Recent studies showed that IBD-induced bone loss was independent upon weight loss.[13] In addition, Card *et al.*[14] reported that the more than half of hip fracture in IBD patients was not due to the steroid use. Inflammation may be the main determinant of bone loss in IBD patients.[6,13] The cytokines released by the inflammatory cells, especially for TNFα can stimulate osteoclast formation and activity, which cause excessive bone resorption and bone loss,[15] and anti-TNFα therapy can decrease the bone loss.[16,17] In addition, bone formation is also inhibited in IBD patients,[18] particularly in children.[13]

Compounds derived from natural products, such as Asian herbs, can protect against bone loss by inhibiting osteoclast formations.[19–22] Recent studies also indicated that DHA[21] and artemisinin,[23–25] antimalaria drug, can inhibit estrogen deficiency- or lipopolysaccharide-induced bone loss by suppressing osteoclast formation or inducing osteoclast apoptosis. Compounds derived from natural products are also useful for the treatment of IBD.[26] Previous studies indicated that Artesunate treatment decreased the high TNFα level and the colon damage induced by dextran sodium sulfate (DSS).[27] However, the effect of DHA on IBD and IBD-induced bone loss is not known. In addition, the overactivation of osteoclasts is a critical way in IBD-induced bone destruction.[28,29] In the present study, we aimed to investigate the role of DHA in IBD-induced bone loss in an adult rat model and explored the potential mechanisms from the aspect of bone formation and bone-resorbing osteoclasts.

Materials and methods

Experimental design

Thirty-two 12-week-old male Sprague-Dawley rats weighing 360–372 g were fed in the specific pathogen-free animal facilities and maintained under conventional conditions ($21 \pm 1^{\circ}C$, 50–80% relative humidity) in a 12 h light–12 h dark cycle. All rats were allowed free access to standard laboratory food and water. After one-week acclimatization to laboratory conditions, the animals were randomly divided into four groups of eight mice: a non-IBD control group, IBD group, and two DHA treatment groups. The dose of DHA for antimalarial treatment was 60 mg/d. The equivalent dose ratio of human to rat is 6.3. Therefore, the calculated dose for rat is 60 mg/60 kg $\times$ 6.3 = 6.3 mg/kg. Therefore, the two doses, 10 and 20 mg/kg which were close to the estimated dose were adopted. The protocol employed here was approved by the Animal Care Committee of Affiliated Hospital of Guangdong Medical University.

Preparation of IBD models and DHA treatment

The chronic IBD model was established as described in previous studies by using DSS (molecular weight 36,000–50,000 MW).[30,31] Briefly, the rats were exposed to 2.5% DSS for five days and then had free access to normal water for one week. Next, the same procedure was performed as above once again. Then, the rats had free access to normal water for four weeks. Control rats were given normal water. For the two treatment groups, the DHA (10 and 20 mg/kg) was given at the second week for a total of five weeks (five times per week). The rats in IBD and control group were also intraperitoneally injected with 3% DMSO five times per week. The data of body weight were obtained every week.

Sample collection

At the sixth week, all rats were sacrificed by anesthesia (7% chloral hydrate, 0.5 mL/100 g body weight). We collected the blood from carotid artery without anticoagulant and it was centrifuged for serum isolation. The serum was divided into aliquots and stored at $-80^{\circ}C$ until analysis. The colon was collected for hematoxylin and eosin (HE) staining. Lumbar spines were collected for bone mineral density (BMD) determination after removing excess soft tissues. Left tibia was harvested for histological examination after decalcified by 10% EDTA. The right tibia was obtained for microCT analysis.

Colitis evaluation

Colon was fixed with 10% formalin solution for 24 h and embedded in paraffin. Five micrometer sections were obtained for HE staining. The severity of colitis was evaluated by using disease activity index (DAI) as previous studies described.[31,32] The DAI includes degree of inflammation (0: none; 1: mild; 2: moderate; 3: severe) and mucosal damage (0: none, 1: mucous layer, 2: submucosa, 3: muscularis and serosa), crypt damage (0: none, 1: basal, 1/3: damaged, 2: basal, 2/3: damaged, 3: entire crypt damaged, 4: epithelium lost), and range of lesions (0: none, 1: 0–25%, 2: 26–50%, 3: 51–75%, 4: 76–100%). All the parameters were summed.

Bone densitometry and microCT analysis

Lumbar spines were wrapped in tissue soaked in normal saline chloride. The BMD was determined by using dual energy X-ray absorptiometry (Hologic Discovery Wi, USA) with small animal software.

Excess soft tissue was removed from tibias and wrapped in tissue soaked in normal saline chloride. The samples were placed in Eppendorf tubes to maintain position. The tibia was imaged with a microCT system (GE Healthcare, eXplore Locus, USA). The imaging parameters were as following: X-ray tube potential of 80 kV, X-ray intensity of 350 μA, field of view 3.0 cm, and 45 μm isotropic resolutions. Tibia images were then reconstructed using software

with a constant threshold value. The 3D images were loaded into the analysis program for further analysis. The following parameters were analyzed: bone volume fractions (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp). The region of interest was set at 1 mm below the growth plate and extending a further longitudinal distance of 2 mm in the distal direction.

Bone histologic assay

Left tibia decalcified by 10% EDTA, dehydrated through a series of ascending ethanol solution (40–100%) and embedded in paraffin. Five micrometer sections were obtained. The sections were dewaxed by using dimethylbenzene and then hydrating by passage through 100–40% ethanol solution. The osteoclasts in bone tissues were identified by tartrate-resistant acid phosphate (TRAP) staining which was performed by using a commercial kit (Sigma 387-A, St Louis, USA). Then, the sections were viewed with a microscope. TRAP-positive length/bone surface were analyzed by using imaging software.

In addition, receptor activator of nuclear factor (NF)-kB ligand (RANKL) expression in bone was determined by immunohistochemical methods. After dewaxing in dimethylbenzene and rehydrating in PBS, the sections were heated in retrieval solution. Then, the sections were incubated in 1% H_2O_2. Subsequently, they were incubated with primary antibodies (Mouse monoclonal anti-RANKL, 1:100 dilutions, Catalog no. sc-59982, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) overnight at 4°C, following incubation with secondary antibodies (1:1000, Catalog no. ab193651, Abcam, MA, USA) at room temperature. Finally, the antigen–antibody complex was visualized by 3,30-diaminobenzidine kit. The RANKL expression was evaluated by the positive area (%) by using the following equation= the area of RANKL-positive growth plate divided by the area of growth plate.

Biochemical marker assay

Serum tumor necrosis factor α (TNFα, Catalog no. RTA00, R&D Systems), RANKL (Quantikine colorimetric sandwich ELISA; Catalog no. MTR00, R&D Systems), tartrate-resistant acid phosphatase 5b (Tracp5b) (Rat TRAP Assay, Catalog no. SB-TR201A, IDS, UK), and procollagen type 1 N-terminal propeptide (P1NP) (Catalog no. AC-33F1, IDS, UK) were determined by using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions. The detection limits were both 5.0 pg/mL for TNFα and RANKL. The intraassay and interassay were both lower than 6%. For Tracp5b, the manufacturer supplied a standard sample (1.9 U/L) and the obtained result in our laboratory was 1.7–1.9 U/L.

In addition, the alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine were determined by using commercial kit (Jiancheng Bio Inc., Nanjing, China; Catalog no. c010-3, c009-3, c013-2, c011-2).

Reverse transcription polymerase chain reaction in vivo

Tibias were crushed under liquid nitrogen and total RNA was extracted by using Trizol reagent. Furthermore, cDNA was synthesized by reverse transcription using a using a Quantscript RT kit (Catalog no. KR103, Tiangen Biotech Co., China). The individual cDNA species were amplified in a reaction mixture containing cDNA aliquot, the relevant sense and antisense primers (RANK: forward 5′ccag gacagggctgatgagaa′3, reverse 5′tggctgacatacaccacgatga′3, TRAF6: 5′agcccacgaaagccagaagaa′3, reverse 5′cccttatg gatttgatgatga′3, Fra-1: 5′agagctgcagaagcagaagg′3, reverse 5′caagtacgggtcctggagaa′3; NFATc1: forward 5′-ctcaccacagggctcactatg-3′, reverse 5′-ttcttcctcccgatgtccgt-3′), and SYBR Premix Ex Taq Mix(Takara Bio Inc., Otsu, Japan). Reactions were initiated by incubation at 94°C for 5 min, and real-time polymerase chain reaction was performed for 40 cycles. Each cycle consisted of 94°C for 15 s, 60°C for 30 s, and 72°C for 20 s.

Statistical analysis

The data were managed and analyzed by suing SPSS16.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± SD. One-way analysis of variance followed by Student–Newman–Keuls *post hoc* was used to compare the mean values of quantitative variables among groups. The score of histopathological lesions was analyzed by Mann–Whitney U test. Spearman correlation analysis was used to show the association between disease score and bone microstructural parameters. P value below 0.05 was regarded as significance of differences.

Results

Body weight

The body weight during the whole experiment is listed in Figure 1. Overall, the body weight of rats in every group increased during the whole experiment. The body weight of 2.5% DSS-treated rat did significantly differ with control

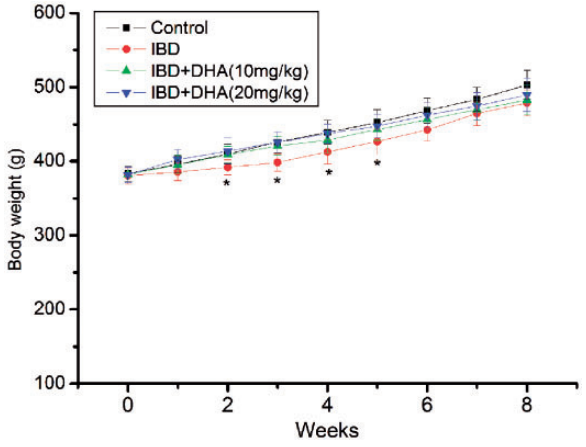

Figure 1. Body weight of rats in control, IBDs, and DHA-treated group. The body weight of 2.5% DSS-treated rat did significantly differ with control rats from the second week (all $p < 0.05$). * control versus IBD, $p < 0.05$. DHA: dihydroartemisinin; IBD: inflammatory bowel disease. (A color version of this figure is available in the online journal.)

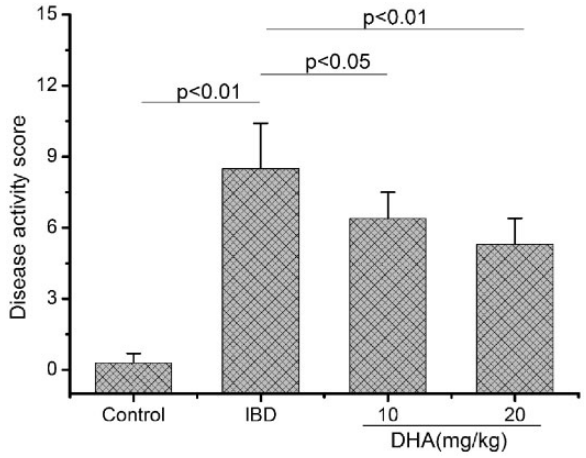

Figure 2. Disease activity score in control, IBDs, and DHA-treated rats. The score of IBD rats was higher than control rats. DHA reduced the damage as shown by the decrease in disease activity score. DHA: dihydroartemisinin; IBD: inflammatory bowel disease.

rats from the second week (all $p < 0.05$). However, the weight loss is less than 10%. The body weights of rats in DHA groups were also lower than control rats, but no significant differences were observed.

Disease activity score

DSS induced IBD in our study as shown in histologic examination and disease activity score (Figure 2). The score of DSS-treated rats was significantly higher than that of control rats ($p < 0.01$). Intervention with DHA obviously reduced the damage as shown by the decrease in disease activity score, in particular at 20 mg/kg ($p < 0.05$).

Serum biomarker

Four biomarkers related with IBD or bone loss were determined in this study (Figure 3). The serum TNFα, Tracp5b, and RANKL were all significantly increased in DSS-treated rats compared with the control (all $p < 0.01$). The treatments with DHA obviously abolished the increase of TNFα, Tracp5b, and RANKL compared with the rats treated with DSS alone. The P1NP level was decreased in IBD rats. The treatments with DHA diminished the decrease of P1NP level caused by IBD, in particular at 20 mg/kg ($p < 0.01$). In addition, AST and ALT were increased in IBD rats compared to the control, but no significant differences in AST, ALT, BUN, and creatinine were observed between IBD rats and those treated with DHA (Table 1).

BMD and bone microstructure

The BMD of DSS-treated rats was obviously decreased compared with that of control rats ($p < 0.05$). The BMD increased in the presence of DHA (Figure 4(a)), in particular at a dose of 20 mg/kg ($p < 0.05$).

Subsequently, we evaluated the bone microstructure by using microCT (Figure 4(b)). Two-dimensional and 3D images both showed the bone loss in DSS-treated rats. The quantitative data further indicated the BV/TV and Tb.N reduce and Tb.Sp increase in DSS rats compared with control (Figure 4(c)). The treatment with DHA protected the rats against bone loss associated with IBD, as shown by increased BV/TV, Tb.N, and decreased Tb.Sp, in particular at a dose of 20 mg/kg (Figure 4(b) and (c)).

We also showed the association between disease score with bone microstructural parameters (Figure 5). The disease score was positively correlated with Tb.Sp ($r = 0.76$) and negatively correlated with BV/TV ($r = 0.92$) and Tb. N ($r = 0.86$).

Bone histological examinations

Histochemical stains showed that TRAP-positive cells were located at bone surface (Figure 6). The TRAP-positive length/bone surface in DSS-treated rats was significantly longer than in control rats ($p < 0.05$). However, the TRAP-positive length/bone surface was decreased in DHA-treated IBD rats compared with IBD group ($p < 0.05$). This result indicated that DHA may protect IBD-induced bone loss by inhibiting osteoclast formation.

Subsequently, we observed the RANKL expression in bone tissues (Figure 7). Control rats showed weak level of RANKL expression. The RANKL expression in DSS-treated rats was markedly increased compared with the control. The RANKL expression was decreased in DHA-treated IBD rats compared with IBD group, in particular at a dose of 20 mg/kg ($p < 0.01$).

mRNA expression

Subsequently, we examined the influence of DHA on the *RANK*, *TRAF6*, *Fra-1*, and *NFATc1* mRNA (Figure 8) expression. The *RANK*, *TRAF6*, *Fra-1*, and *NFATc1* expression in IBD rats were significantly higher than the control ($p < 0.01$). The DHA treatment dose dependently abolished the up-regulation of these mRNA expressions in IBD rats ($p < 0.05$).

Discussion

Bone loss induced by IBD is the major extra-intestinal cause of morbidity.[33] Low bone mass and osteoporosis can occur in about 50% of IBD patients.[8,33] Compounds derived from natural products have great potential for the treatment of IBD.[26] However, its role in IBD-induced bone loss is not completely clarified. In the present study, we showed that DHA reduced the colon damage induced by DSS. Moreover, we found that DHA also protected against the bone loss in IBD rats via promoting bone formation and inhibiting osteoclastic bone resorption.

Many agents or drugs have been adopted to treat IBD, including steroid and anti-TNFα monoclonal antibody. However, they also have significant adverse effects.[34] More and more studies indicated that natural products and herbal medicines have exhibited efficacy for IBD in animal model or clinical trials.[26] DHA and artemisinin, antimalaria drug, have been found to have potential to inhibit cancer cells and inflammation via modulating the NF-κB pathway.[35–37] NF-κB is also a critical transcription factor in inflammatory process.[38] In addition, a previous study indicated that artesunate was available as a potential

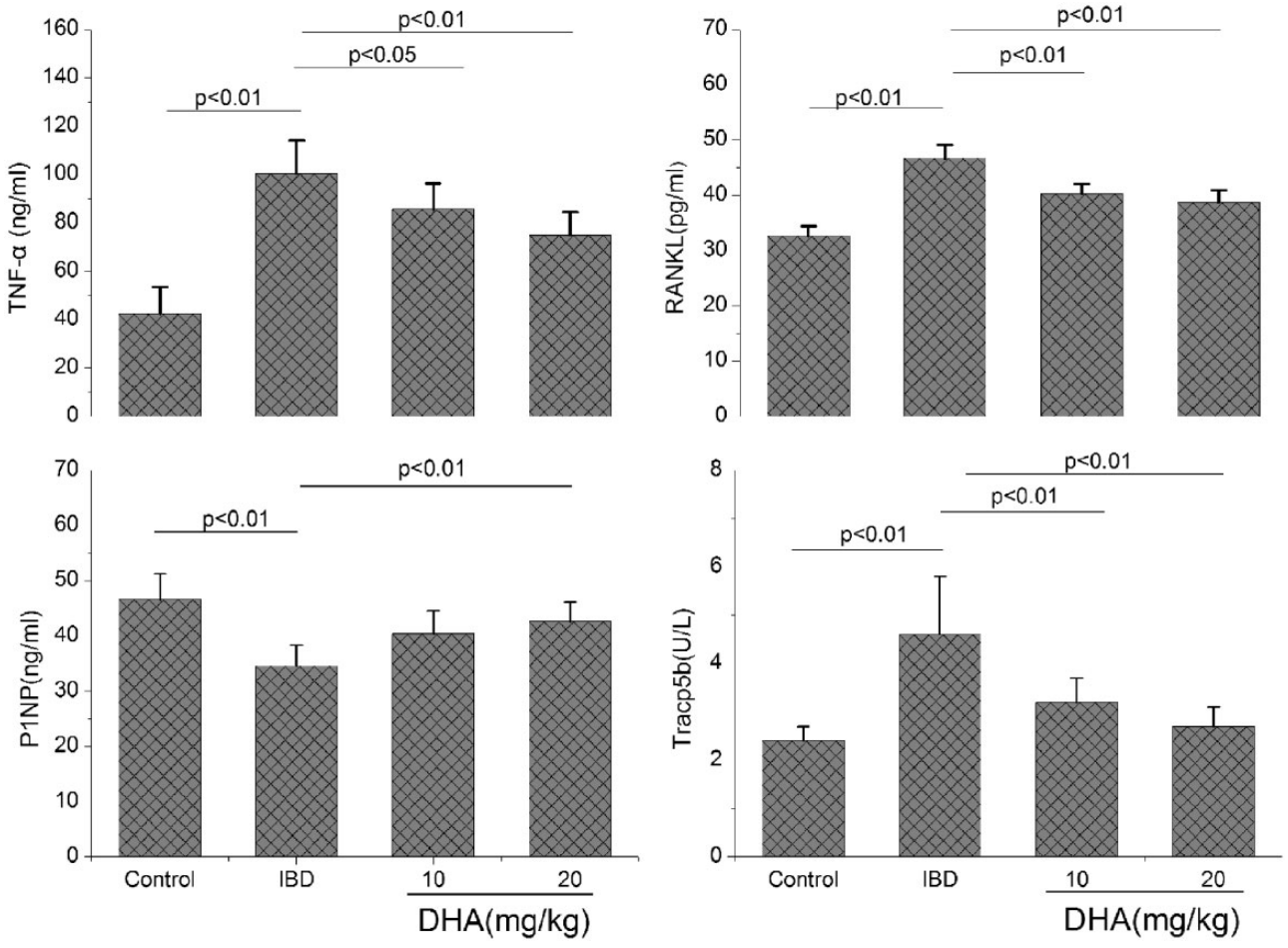

Figure 3. Serum TNFα, RANKL, tartrate-resistant acid phosphatase 5b (Tracp5b), and P1NP levels in control, IBDs, and DHA-treated rats. The serum TNFα, Tracp5b, and RANKL were increased and P1NP was decreased in IBD rats compared with the control. The treatments with DHA obviously abolished the changes of TNFα, Tracp5b, RANKL, and P1NP compared with the rats treated with DSS alone. DHA: dihydroartemisinin; P1NP: procollagen type 1 N-terminal propeptide; RANKL: receptor activator of nuclear factor (NF)-kB ligand; TNFα: tumor necrotic factor α.

Table 1. Liver and renal dysfunction.

	Control	IBD	IBD + DHA (10 mg/kg)	IBD + DHA (20 mg/kg)
ALT (U/L)	42.3 ± 10.6	58.7±11.3*	64.5 ± 12.5	65.7 ± 11.8
AST (U/L)	76.4 ± 11.4	87.9 ± 12.3*	92.3 ± 13.7	94.6 ± 12.4
BUN (mmol/L)	5.7 ± 1.5	5.9 ± 1.3	5.9 ± 1.4	6.2 ± 1.7
Creatinine (umol/L)	24.6 ± 4.3	25.3 ± 3.8	26.4 ± 2.4	26.8 ± 3.7

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; DHA: dihydroartemisinin; IBD: inflammatory bowel disease.
*$p < 0.05$ versus control.

therapy for IBD.[27] Therefore, we hypothesize that DHA may have the same role. In this study, we demonstrated that DHA administration significantly reduced the colon damage induced by DSS. Further studies showed that DHA inhibited the serum TNFα which was a critical cytokine in IBD. Previous several studies have shown that TNFα inhibition is effective in reducing the severity of IBD.[9,34] Our data indicated that DHA may abolish the colon damage induced by DSS through suppression of TNFα production.

Previous studies have shown that DHA inhibited RANKL-induced NF-κB activity[21,25] and osteoclast formation[21,25] or promoted osteoclast apoptosis.[39] Therefore, we speculate that DHA may also protect against bone loss induced by IBD. Interestingly, bone loss was reduced in IBD rats treated with DHA, as shown by improvement in BMD and bone microstructure parameters. To the best of our knowledge, few studies have observed the effects of natural products on IBD and IBD-induced bone loss together. Our data indicate that DHA not only have potential to treat IBD, but also inhibit IBD-induced bone loss.

TNFα plays one of key roles in bone loss in IBD patients.[13,16,17] TNFα can inhibit bone formation via

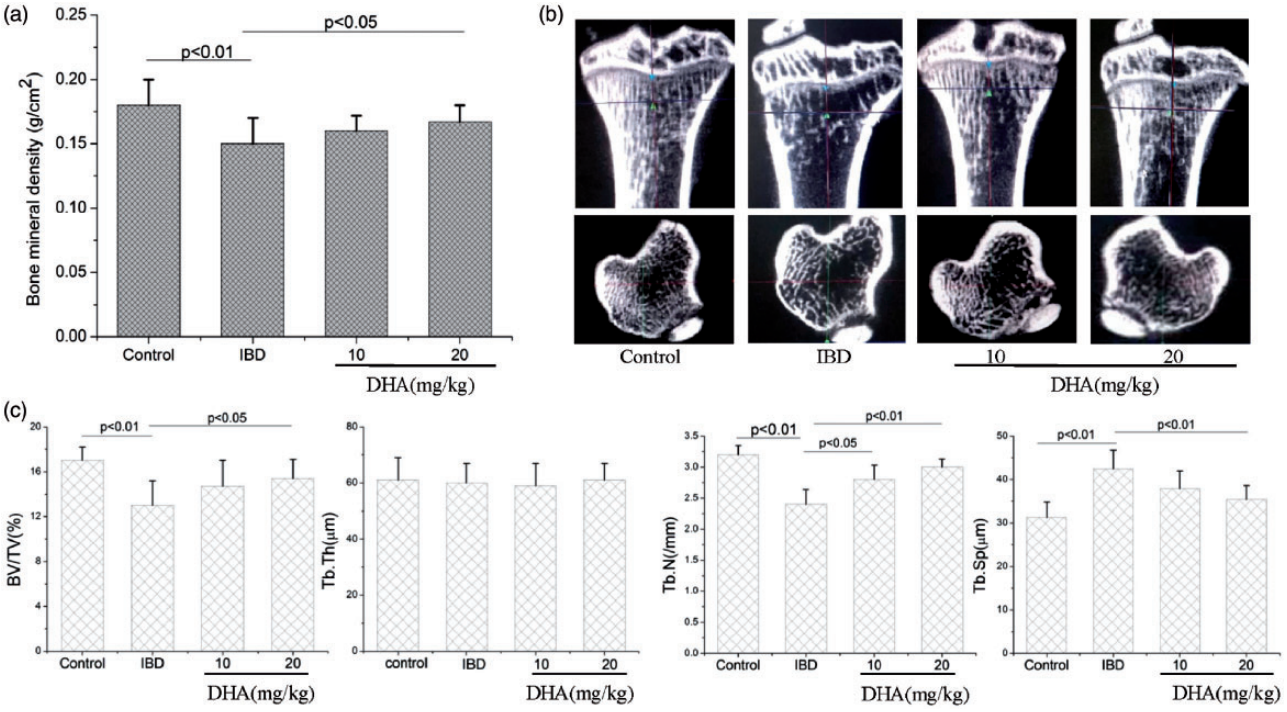

Figure 4. BMD (a) and bone microstructure (b, c) in control, IBDs, and DHA-treated rats. (b) MicroCT image of tibia; (c) quantitative analysis of BV/TV, Tb.N, Tb.Th, and Tb.Sp. The BMD, BV/TV, and Tb.N of IBD rats were decreased and Tb.Sp was increased compared to the control. The treatments with DHA obviously abolished these changes. BV/TV: bone volume fractions; DHA: dihydroartemisinin; IBD: inflammatory bowel disease; Tb.N: trabecular number; Tb.Sp: trabecular separation; Tb.Th: trabecular thickness.

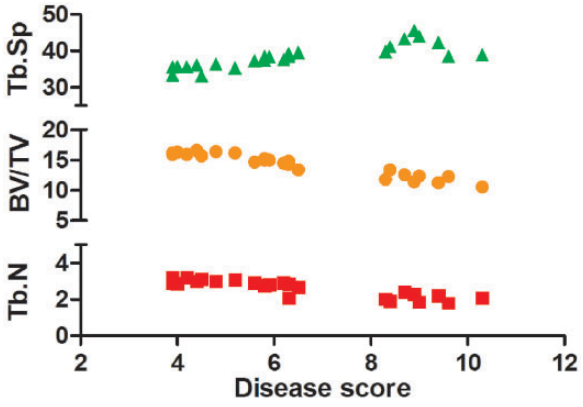

Figure 5. The correlations between disease score and bone microstructural parameters. The disease score was positively correlated with Tb.Sp ($r = 0.76$) and negatively correlated with BV/TV ($r = 0.92$) and Tb.N ($r = 0.86$). BV/TV: bone volume fractions; Tb.N: trabecular number; Tb.Sp: trabecular separation. (A color version of this figure is available in the online journal.)

suppression of osteoblast.[40,41] TNFα suppresses serum insulin-like growth factor-1 which is a stimulator of bone formation.[41] A recent study showed that osteoblast activity was suppressed in DSS-treated mice.[13] In our study, inhibition in bone formation was also observed in IBD rats as shown by the decrease of P1NP which is a biomarker of bone formation. DHA treatment can attenuate the inhibition in bone formation. Zhou *et al.*[21] showed that DHA did not affect formation, differentiation, and mineralization of osteoblast. Therefore, the improvement in bone formation in IBD rats may be due to the decrease of TNFα. In addition, TNFα may stimulate RANKL expression or cooperate with RANKL to stimulate osteoclast formation.[42,43] RANKL is the critical cytokine associated with osteoclasts formation and differentiation.[44,45] In the present study, excessive osteoclast formation was observed in IBD rats. In addition, our data showed that DHA intervention inhibited IBD-induced osteoclast formation and high RANKL expression in bone tissues. Our data also showed that osteoclast formation-related gene expressions, including *RANK*, *TRAF6*, *Fra-1*, and *NFATc1*, were increased in bone tissues of IBD rats. DHA treatment significantly inhibited those gene expressions. Hotokezaka *et al.*[46] showed that TNFα induced TRAP-positive mononuclear cells fusion in the absence of RANKL. We speculated that the decrease in osteoclast formation may be due to the inhibition of RANKL/RNAK signal pathway by DHA or by the decrease of TNFα. In addition, the decrease of TNFα will reduce intestinal inflammation. Consequently, bone loss will be reduced. However, Irwin *et al.*[13] showed percentage of osteoclast surface or markers of osteoclast maturation were not increased in DSS-treated mice which indicated that inflammation predominantly affected osteoblast activity. A young mice IBD model in which bone formation was predominant was used in their study. However, we established an adult rat model in which bone formation and resorption were both active in the present study.

There are several limitations in our study. First, several chemical compounds have been used to induce colitis, such as 2,4-dinitrobenzene sulfonic acid and acetic acid. Only DSS-induced IBD was used in our study. Second, the focus of our study is IBD-induced bone loss. The therapeutic effect of DHA on IBD is just primarily observed.

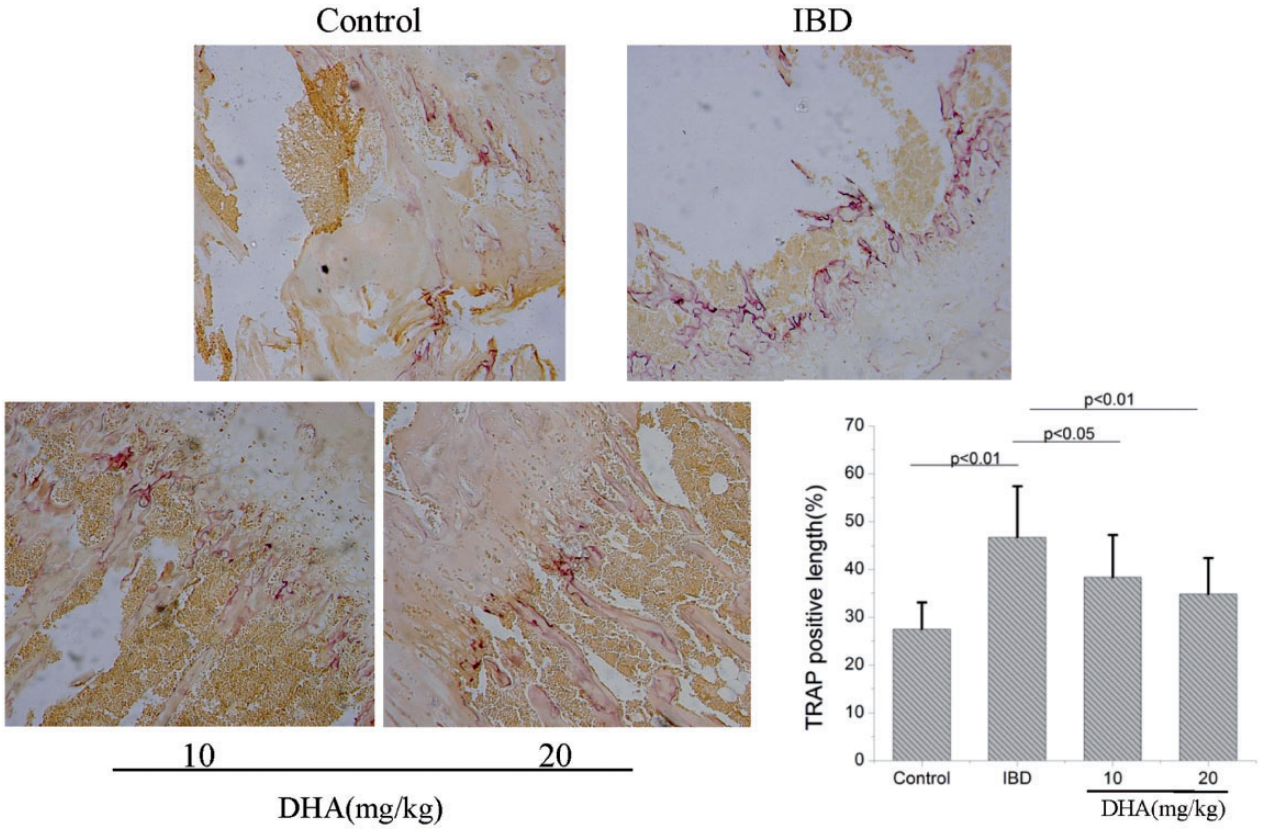

Figure 6. Histochemical stains show TRAP-positive cells in control, IBDs, and DHA-treated rats. The TRAP-positive length/bone surface in IBD rats was significantly longer than control rats. DHA treatment inhibited the osteoclast formation induced by IBD. DHA: dihydroartemisinin; IBD: inflammatory bowel disease; TRAP: tartrate-resistant acid phosphate. (A color version of this figure is available in the online journal.)

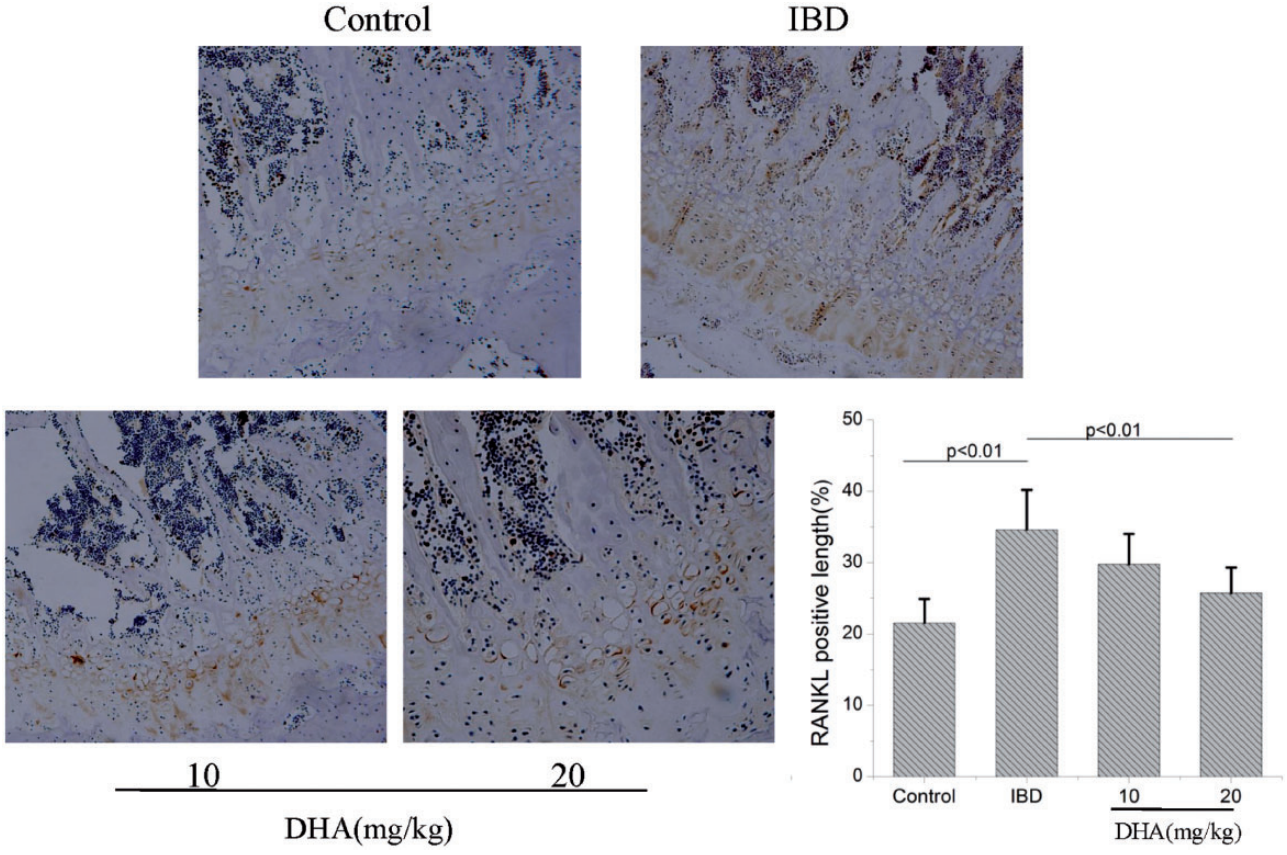

Figure 7. Immunochemical stains show RANKL expression in control, IBDs, and DHA-treated rats. The RANKL-positive length in IBD rats was significantly longer than control rats. DHA treatment inhibited the RANKL expression induced by IBD. DHA: dihydroartemisinin; IBD: inflammatory bowel disease; RANKL: receptor activator of nuclear factor (NF)-kB ligand. (A color version of this figure is available in the online journal.)

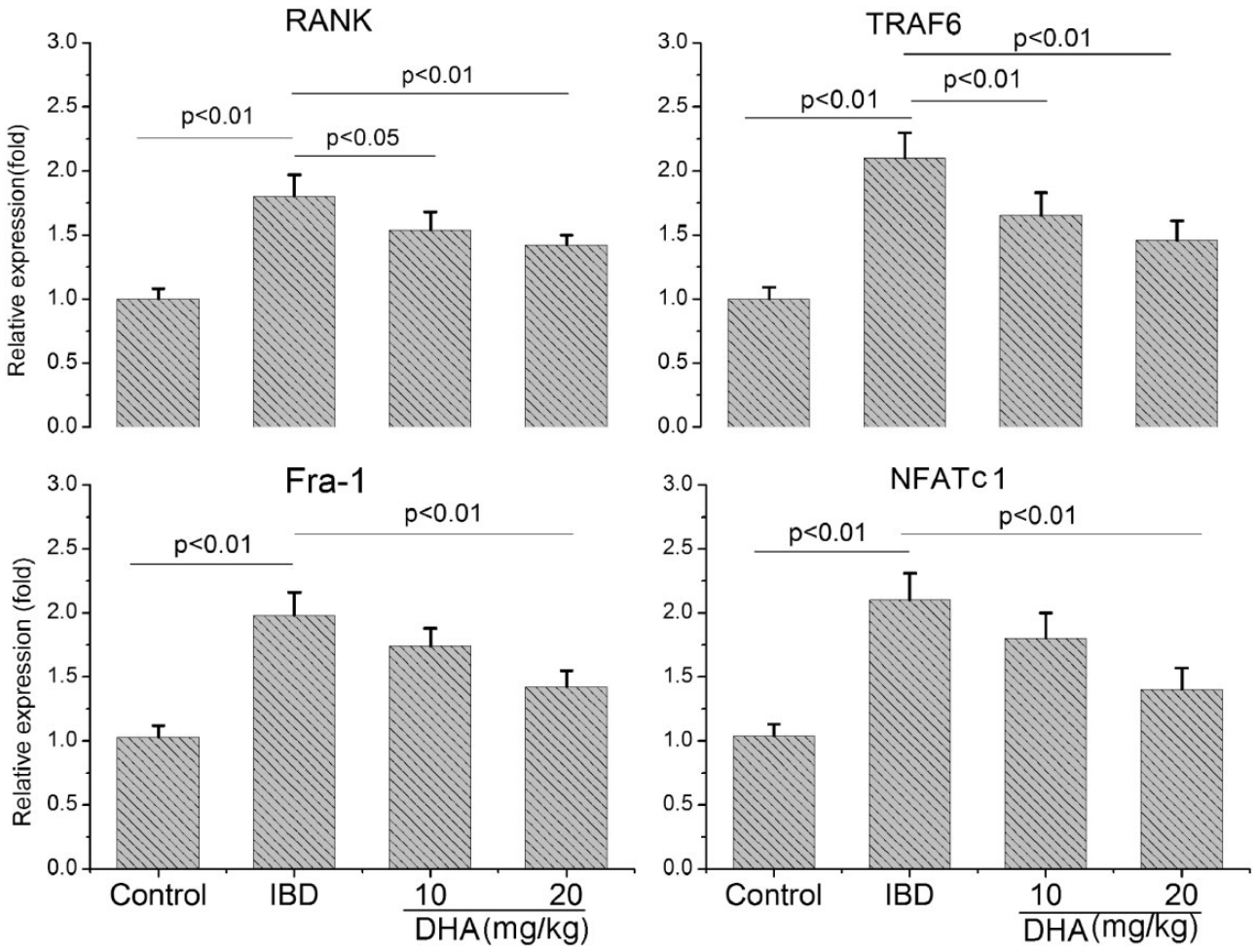

Figure 8. The mRNA expression of RANK, TRAF6, Fra-1, and NFATc1 in control, IBD and DHA-treated rats. The RANK, TRAF6, Fra-1, and NFATc1 expression in IBD rats were higher than the control. The DHA treatment abolished the up-regulation of these mRNA expressions in IBD rats. DHA: dihydroartemisinin; IBD: inflammatory bowel disease; RANK: receptor activator of nuclear factor (NF)-kB.

Third, whether the roles of DHA on IBD-induced bone loss are mediated by TNFα or by its direct effects on osteoclast need further study. In addition, other cytokine related to bone loss in IBD, such as IL-17 and interferon γ, was not evaluated in this study. Finally, for a therapeutic study, the sample sizes were relatively small and the dose–response relationships were not observed.

In conclusion, our data indicated that DHA may protect against DSS-induced IBD and IBD-induced bone loss. DHA suppresses the osteoclast formation in bone of IBD rats. The protective role may be due to its inhibitory effects on TNFα or RANKL. DHA may be a potential therapeutic agent for IBD and IBD-induced bone loss.

Authors' contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; XG, ZC, ZX performed experiments; XG, ZC, and FL analyzed data; XG and ZC prepared figures; XG and ZC drafted manuscript; KZ and YY edited and revised manuscript. XG and ZC contributed equally to this paper.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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(Received January 16, 2018, Accepted March 16, 2018)