Anti-Inflammatory Effect of Selenium-Enhanced Docosahexaenoic Acid in Lipopolysaccharide-Induced Macrophage Inflammatory Response (Postprint)

Authors: Xu Qipin, Huang Yixin, Luo Zhengzhong, Shen Liuhong, Yao Xueping, Yu Shumin, Cao Suizhong

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Abstract

To investigate whether selenium affects the anti-inflammatory effect of docosahexaenoic acid (DHA) in lipopolysaccharide (LPS)-induced inflammatory responses in macrophages. Murine macrophage cell line RAW264.7 cells were treated with 10 g/mL DHA, 10 g/mL DHA + 0.05 mol/L sodium selenite, 1 g/mL LPS, 10 g/mL DHA + 1 g/mL LPS, or 10 g/mL DHA + 1 g/mL LPS + 0.05 mol/L sodium selenite for 24 h, with an untreated control group established simultaneously. Semi-quantitative reverse transcription PCR was used to detect the mRNA expression levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-10 (IL-10) in cells, and enzyme-linked immunosorbent assay (ELISA) was used to determine the contents of TNF-α, IL-1β, IL-6, and IL-10 in the culture supernatant. The results showed that the addition of sodium selenite (0.05 mol/L) not only significantly or highly significantly enhanced the inhibitory effect of DHA (10 g/mL) on LPS (1 g/mL)-induced pro-inflammatory cytokine TNF-α mRNA expression ($P<0.05$) and IL-1β production ($P<0.01$) in RAW264.7 cells, but also highly significantly enhanced the effect of DHA in promoting anti-inflammatory cytokine IL-10 mRNA expression ($P<0.01$). This suggests that selenium can enhance the anti-inflammatory effect of DHA in LPS-induced macrophage inflammatory responses.

Full Text

Selenium Enhances the Anti-Inflammatory Effects of Docosahexaenoic Acid in Lipopolysaccharide-Induced Inflammatory Responses in Macrophages

XU Qipin, HUANG Yixin, LUO Zhengzhong, SHEN Liuhong, YAO Xueping, YU Shumin, CAO Suizhong*

College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China

Abstract

To investigate whether selenium (Se) influences the anti-inflammatory effects of docosahexaenoic acid (DHA) in lipopolysaccharide (LPS)-induced inflammatory responses in macrophages, RAW264.7 mouse macrophage cells were treated for 24 hours with 10 μg/mL DHA, 10 μg/mL DHA + 0.05 μmol/L sodium selenite, 1 μg/mL LPS, 10 μg/mL DHA + 1 μg/mL LPS, or 10 μg/mL DHA + 1 μg/mL LPS + 0.05 μmol/L sodium selenite, with a normal control group receiving no treatment. Semi-quantitative reverse transcription PCR was used to detect mRNA expression levels of tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6), and interleukin 10 (IL-10), while enzyme-linked immunosorbent assay (ELISA) was employed to measure the concentrations of TNF-α, IL-1β, IL-6, and IL-10 in culture supernatants. The results demonstrated that supplementation with sodium selenite (0.05 μmol/L) not only significantly or highly significantly enhanced the inhibitory effects of DHA (10 μg/mL) on LPS (1 μg/mL)-induced expression of the pro-inflammatory cytokine TNF-α mRNA ($P<0.05$) and production of IL-1β ($P<0.01$) in RAW264.7 cells, but also highly significantly enhanced DHA' s promoting effect on anti-inflammatory cytokine IL-10 mRNA expression ($P<0.01$). These findings suggest that selenium can enhance the anti-inflammatory effects of DHA in LPS-induced inflammatory responses in macrophages.

Keywords: Docosahexaenoic acid; selenium; macrophages; anti-inflammatory effect

Introduction

Excessive or persistent inflammatory responses can trigger numerous diseases, and effectively controlling and treating inflammatory diseases remains a major research focus. Overexpression of cytokines such as tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) can lead to excessive inflammatory reactions [1], and inhibiting the release of these pro-inflammatory mediators may help attenuate inflammatory responses and improve disease prognosis. Studies have found that long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs) such

as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from dietary sources influence lipoprotein metabolism, endothelial cell function, vascular reactivity, and the production of inflammatory markers and cytokines, contributing to the treatment of inflammatory diseases such as cardiovascular disease in humans [2]. Selenium (Se) is an essential trace element for animals that is incorporated into selenoproteins in the form of selenocysteine and participates in the regulation of oxidative stress, redox processes, and other important cellular processes in almost all tissues and cells, thereby exerting anti-inflammatory effects [3-4]. Dietary intake of adequate selenium can improve various inflammatory diseases including mastitis, cardiovascular disease, and osteoporosis [5-7]. While research on the individual anti-inflammatory effects and mechanisms of DHA and selenium has made significant progress, studies investigating the interaction between n-3 PUFAs and selenium remain limited.

Macrophages play crucial roles in the initiation, development, and resolution of inflammation [8], and macrophages activated by lipopolysaccharide (LPS) produce large amounts of pro-inflammatory cytokines including IL-1β, TNF-α, and interleukin 6 (IL-6) [8-9]. Studies have shown that DHA has a stronger effect than EPA in reducing LPS-induced expression of pro-inflammatory cytokines IL-1β and IL-6 in macrophages [10-12]. Therefore, based on the commonly used macrophage cell line—RAW264.7 mouse macrophages—this study investigated whether selenium influences the anti-inflammatory effects of DHA in LPS-induced inflammatory responses by monitoring mRNA expression and protein levels of pro-inflammatory and anti-inflammatory cytokines, aiming to provide a theoretical basis for alternative strategies to modulate uncontrolled inflammatory responses through dietary intervention.

Materials and Methods

1.1 Cell Line

Mouse (Mus musculus) macrophage cell line RAW264.7 was purchased from the Shanghai Institute of Life Sciences, Chinese Academy of Sciences.

1.2 Reagents

DMEM high-glucose culture medium was purchased from Gibco; dimethyl sulfoxide (DMSO), LPS (L2880), and sodium selenite (Na2SeO3, S5261) were all purchased from Sigma; DHA (S49140, purity $\$ 98 , IL - 1 \$$, IL-6, and interleukin 10 (IL-10) biotin double-antibody sandwich ELISA kits were purchased from Beijing Yanda Biotechnology Co., Ltd.

1.3 Equipment

CO2 incubator and microplate reader (Thermo); ultra-clean workbench; PCR thermal cycler and gel imaging system (Bio-Rad); DYY-III electrophoresis apparatus (Beijing Liuyi Instrument Factory); and other equipment.

1.4.1 Cell Culture and Grouping

RAW264.7 mouse macrophage cells were seeded into culture dishes (10 cm diameter) and cultured in DMEM high-glucose medium (containing 10% FBS) at 37°C with 5% CO2 until reaching 80% confluence. The experimental groups are shown in Table 1 , with three replicates per group. Cells were seeded into 6-well plates at a density of 4.5$×$10^5 cells per well, with 2 mL of DMEM high-glucose medium (containing 10% FBS) added to each well, and cultured at 37°C with 5% CO2 for 24 h. The medium was then replaced with fresh medium, and LPS (100 μg/mL), DHA (10 mg/mL), and sodium selenite (100 μmol/mL) were added according to group assignments to achieve final concentrations of 1 μg/mL LPS, 10 μg/mL DHA, and 0.05 μmol/L sodium selenite, respectively. Cells were cultured for an additional 24 h, after which cells and culture supernatants were collected separately. Cells were used for total RNA extraction, and supernatants were stored at -20°C.

1.4.2 Semi-quantitative Reverse Transcription PCR Detection of TNF-α, IL-1β, IL-6, and IL-10 mRNA Expression

Total RNA was extracted from cells in each group using the EASYspin plus tissue/cell RNA rapid extraction kit according to the manufacturer' s instructions. First-strand cDNA was synthesized using the Fast Quant cDNA first-strand synthesis kit according to the manufacturer' s instructions and stored at -20°C. Using the first-strand cDNA as template, PCR amplification was performed with respective primers (synthesized by BGI, with information provided in Table 2). The reaction system consisted of: 1 μL cDNA, 1 μL each of forward and reverse primers, 9.5 μL Premix Taq (TaKaRa Taq™ Version 2.0 plus dye), and 7.5 μL ddH2O, for a total volume of 20 μL. Reaction conditions were: denaturation at 94°C for 30 s, annealing at the appropriate temperature for 30 s, extension at 72°C for 1 min, for 30 cycles, with a final extension at 72°C for 10 min, and storage at 4°C.

PCR products were subjected to 2% agarose gel electrophoresis (100 V, 30 min) and images were captured using the Bio-Rad gel imaging system. Electrophoresis images were analyzed using Image J software to calculate the gray values of each band. β-actin was used as the internal reference, and the mRNA expression level of the target gene was calculated as: (gray value of target gene band/gray value of reference gene band) × 100.

1.4.3 ELISA Determination of TNF-α, IL-1β, IL-6, and IL-10 Content

The concentrations of TNF-α, IL-1β, IL-6, and IL-10 in cell culture supernatants were determined using mouse TNF-α, IL-1β, IL-6, and IL-10 biotin double-antibody sandwich ELISA kits according to the manufacturer' s instructions. Each concentration standard and sample was tested in triplicate, with blank wells used for zeroing. A microplate reader was used to sequentially measure the optical density (OD) values of each well at 450 nm wavelength. Standard

curves were plotted separately to calculate cytokine concentrations in samples.

1.5 Statistical Analysis

All data are presented as "mean ± standard deviation" and were analyzed using SPSS 20.0 software. One-way ANOVA was used for data analysis, with $P<0.05$ considered statistically significant.

Results

2.1 Effects of DHA and Se on LPS-Induced TNF-α, IL-1β, IL-6, and IL-10 mRNA Expression Levels

Semi-quantitative reverse transcription PCR results (Figure 1 [Figure 1: see original paper]) showed that treatment with DHA (10 μg/mL) or DHA (10 μg/mL) plus sodium selenite (0.05 μmol/L) had no significant effect on TNF-α, IL-1β, IL-6, and IL-10 mRNA expression levels in cells not induced by LPS ($P>0.05$). Treatment with DHA (10 μg/mL) highly significantly reduced LPS (1 μg/mL)-induced TNF-α and IL-6 mRNA expression levels ($P<0.01$), significantly increased IL-10 mRNA expression ($P<0.05$), and showed a trend toward reduced IL-1β mRNA expression, though the difference was not significant ($P>0.05$). Supplementation with sodium selenite (0.05 μmol/L) significantly enhanced the inhibitory effect of DHA (10 μg/mL) on LPS (1 μg/mL)-induced TNF-α mRNA expression ($P<0.05$) and highly significantly enhanced DHA' s promoting effect on LPS (1 μg/mL)-induced IL-10 mRNA expression ($P<0.01$), while showing trends toward reduced IL-6 and IL-1β mRNA expression, though these differences were not significant ($P>0.05$). Combined treatment with DHA (10 μg/mL) and sodium selenite (0.05 μmol/L) significantly downregulated LPS-induced IL-1β mRNA expression ($P<0.05$).

2.2 Effects of DHA and Se on LPS-Induced Production of TNF-α, IL-1β, IL-6, and IL-10

ELISA results (Figure 2 [Figure 2: see original paper]) showed that treatment with DHA (10 μg/mL) or DHA (10 μg/mL) plus sodium selenite (0.05 μmol/L) had no significant effect on TNF-α, IL-1β, IL-6, and IL-10 concentrations in cells not induced by LPS ($P>0.05$). Treatment with DHA (10 μg/mL) significantly or highly significantly reduced LPS (1 μg/mL)-induced production of the pro-inflammatory cytokines IL-1β ($P<0.05$), TNF-α ($P<0.01$), and IL-6 ($P<0.01$), while significantly increasing the anti-inflammatory cytokine IL-10 concentration ($P<0.05$). Supplementation with sodium selenite (0.05 μmol/L) highly significantly enhanced the effect of DHA (10 μg/mL) in reducing LPS (1 μg/mL)-induced IL-1β production ($P<0.01$), while showing trends toward reduced TNF-α and IL-6 concentrations and increased IL-10 concentration, though these differences were not significant ($P>0.05$). Combined treatment with sodium selenite (0.05 μmol/L) and DHA (10 μg/mL) highly significantly increased IL-10 concentration in LPS (1 μg/mL)-induced cells ($P<0.01$).

Discussion

Cytokine production is important for controlling the growth and spread of invading pathogens; however, excessive cytokines are detrimental to the organism. For example, persistent overproduction of TNF-α and IL-1β causes muscle wasting and bone loss [9], and regulating inflammatory mediator release can improve disease prognosis. Studies have shown that incubating cells with EPA and DHA can inhibit IL-6 and TNF-α production in RAW264.7 cells [13] and suppress LPS-induced TNF-α and IL-1β mRNA expression in THP-1 cells [14]. Selenium exerts immunomodulatory effects through selenoproteins, and selenium supplementation can inhibit the mitogen-activated protein kinase pathway to significantly reduce LPS-induced cyclooxygenase-2 (COX-2) and TNF-α gene expression in macrophages [15], while TNF-α gene expression is significantly increased in selenoprotein-deficient macrophages [16].

The present study showed that DHA (10 μg/mL) highly significantly inhibited LPS (1 μg/mL)-induced TNF-α and IL-6 mRNA expression in RAW264.7 cells at 24 h, significantly promoted IL-10 mRNA expression, but showed no significant effect on downregulating IL-1β mRNA expression. Sodium selenite (0.05 μmol/L) significantly enhanced the inhibitory effect of DHA (10 μg/mL) on LPS (1 μg/mL)-induced TNF-α mRNA expression and the promoting effect on IL-10 mRNA expression in macrophages at 24 h. Additionally, combined treatment with DHA (10 μg/mL) and sodium selenite (0.05 μmol/L) significantly downregulated LPS (1 μg/mL)-induced IL-1β mRNA expression in macrophages, though sodium selenite (0.05 μmol/L) did not significantly affect DHA' s (10 μg/mL) action in reducing IL-6 mRNA expression.

Furthermore, protein-level detection results showed that DHA (10 μg/mL) significantly or highly significantly reduced IL-1β, TNF-α, and IL-6 concentrations in LPS (1 μg/mL)-induced macrophages at 24 h, while significantly increasing IL-10 concentration. Sodium selenite (0.05 μmol/L) enhanced DHA' s (10 μg/mL) downregulating effect on IL-1β concentration and upregulating effect on IL-10 concentration in LPS (1 μg/mL)-induced macrophages at 24 h.

Thus, supplementation with sodium selenite (0.05 μmol/L) not only significantly enhanced the inhibitory effects of DHA (10 μg/mL) on pro-inflammatory cytokine TNF-α mRNA expression and IL-1β production, but also enhanced DHA' s promoting effects on anti-inflammatory cytokine IL-10 mRNA expression and protein production in RAW264.7 cells induced by LPS (1 μg/mL) for 24 h. These results suggest that selenium can enhance the anti-inflammatory effects of DHA in LPS-induced inflammatory responses.

By influencing the expression of pro-inflammatory cytokines TNF-α and IL-1β and anti-inflammatory cytokine IL-10 mRNA, selenium can enhance the anti-inflammatory effects of DHA in LPS-induced inflammatory responses in macrophages.

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*Corresponding author, professor, E-mail: suizhongcao@126.com

Note: Figure translations are in progress. See original paper for figures.