Microglia response to fatty acid binding protein-UCP2 axis in facilitating neuroinflammation

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BACKGROUND

Introduction

Hypothalamic inflammation contributes to metabolic dysregulation and the onset of obesity [1, 2]. Dietary saturated fatty acids activate microglia via an NFkB mediated pathway to release pro-inflammatory cytokines, resulting in dysfunction or death of surrounding neurons [3, 4]. Fatty acid binding protein (FABP)-4 is a lipid chaperone that regulates metabolic and inflammatory pathways in response to fatty acids [5]. Although inhibiting FABP4 in peripheral macrophages results in reduced obesity-induced inflammation via an uncoupling protein 2 (UCP2)-redox based mechanism [6], the expression of FABP4 and a potential FABP4-UCP2 axis in microglia is largely uncharacterized.

Hypothesis

We hypothesized that inhibition of FABP in microglia would attenuate lipopolysaccharide (LPS) inflammations through a UCP2 mediated mechanism (Figure 1).

Significance

• Chronic activation of microglia in the hypothalamus contributes to various metabolic diseases such as obesity.
• Diets rich in saturated fatty acids induce inflammation, which results in cell death and obesity.
• FABP4-UCP2 axis in microglia could decrease neuroinflammation, offering a novel anti-obesity therapeutic target.

Objectives

• Determine specific FABP isoform gene expression in microglial and brain tissue (Fig 1A-C).
• Determine changes in UCP2, arginase, and inducible nitric oxide synthase (iNOS) expression following inhibition of FABPs in the presence/absence of LPS in microglial (Fig 4A-C).

METHODS

Cell culture and reagents: Immortalized murine microglial (BV2) were grown in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin, streptomycin, and neomycin (Invitrogen) and maintained at 37°C with 5% CO2. Pan-FABP inhibitor (HTS01037,Caymen) was used at a final concentration of 30 μM LPS (Sigma-Aldrich) was used at a concentration of 100 μg/ml in DMEM. [6]

BV2 microglial were seeded in 6 well plates and grown to ~80% confluency. Time points were based on Xu et al. [8]. For the first set of experiments, BV2 were treated with either vehicle control (DMSO). 15 μM or 30 μM HTS01037 for 24 h and collected with TriZol (Invitrogen) and stored at −20°C. For the second set of experiments, BV2 microglial were pretreated with either vehicle control or 30 μM HTS01037 for 3h and challenged with LPS or vehicle for 12 h. Cells were collected using TriZol and stored at −20°C until used.

Real-time qRT-PCR: Total RNA was extracted from BV2 cells with the aid of Trizol and Qiangen RNeasy micro column as previously described [7, 8]. A final concentration of RNA (300 ng/μl) was determined using the 260/280 nm ratio (NanoDrop ND-8000). Gene was determined using qRT-PCR (Roche LightCycler 1.5) with SYBR Green detection. Primer sequences were generated using MacVector 15. Relative RNA levels were normalized to GAPDH using the Δ-ΔCT method [9]. Significant differences were determined using student’s unpaired two-tailed t-test (GraphPad Prism 5). Amplification products were separated via electrophoresis on 3% agarose gels stained with SYBR Green.

RESULTS

Inhibition of FABP in BV2 microglia increases UCP2 expression is dose dependent

Inhibition of FABP trends to reduce iNOS gene expression in microglial

Inhibition of FABP stabilizes LPS induced pro-inflammatory gene expression

REFERENCES


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