In Vitro Evidence of Anti-Inflammatory and Anti-Obesity Effects of Medium-Chain Fatty Acid–Diacylglycerols

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Dietary approaches using structured lipids, including medium-chain fatty acids and diacylglycerols, have been adopted for the prevention of obesity-induced chronic inflammation. In an extension to previous studies, medium-chain fatty acid–diacylglycerol enriched dietary oil (MCDG) was prepared by interesterification of canola oil and medium-chain fatty acid–triacylglycerols. The consequent MCDG product was applied to RAW264.7 macrophages followed by the assessment of multiple inflammatory responses. Compared with conventionally used canola and olive oil controls, MCDG suppressed macrophage phagocytosis, as assessed by the uptake of microsphere beads. Furthermore, the production of IL-6 and TNF-α, transcription of COX-2 and iNOS, and expression of CD80 on cell surfaces were downregulated by MCDG in LPS-stimulated macrophages. Subsequently, differentiated 3T3-L1 adipocytes were evaluated for proinflammatory cytokine production and lipid accumulation. IL-6 production was marginally affected and lipid accumulation was inhibited by MCDG. Taken together, these results suggest that MCDG has potential as an alternative oil for cooking in order to prevent obesity-induced inflammation.

Keywords: Medium-chain fatty acids, diacylglycerols, functional oils, anti-obesity, anti-inflammatory

Introduction

Multiple dietary approaches have been attempted to evaluate the effects of various lipids on obesity and lipid metabolism [1–4]. Among these, medium-chain fatty acids (MCFAs), consisted of 6–12 carbons, mostly saturated, have been reported to exert anti-inflammatory and anti-obesity effects [4, 5]. Compared with conventional long-chain fatty acids (LCFAs), MCFAs do not require carnitine palmitoyl transferase for mitochondrial transport in cellular energy production. Consequently, MCFAs are rapidly oxidized to acetyl-CoA via β-oxidation [6, 7], resulting in increased energy expenditure, reduced fat mass, and downregulated adipogenic gene expression compared with LCFAs [8, 9].

With regard to fatty acid composition on the glycerol backbone of dietary lipids, diacylglycerols (DAGs) exhibited potent suppression in lipid accumulation compared with triacylglycerols (TAGs). Studies have revealed that, during intestinal digestion, TAGs are hydrolyzed to two free fatty acids (FFAs) and 2-monoacylglycerol (2-MG) by pancreatic lipase, followed by absorption in intestinal epithelial cells. TAGs are re-synthesized from absorbed 2-MG and FFAs by monoacylglycerol acyltransferases (MGATs) and diacylglycerol acyltransferases (DGATs) in the epithelial cells. In contrast, dietary 1,3-DAG was shown to be hydrolyzed to 1-MG and FFA by lipase and absorbed into intestinal cells. Absorbed 1-MG and FFA are not synthesized to TAG owing to the lack of 1-MG-specific MGAT in humans [10–12]. Therefore, retarded re-acylation of 1-MG to TAG prevents postprandial hyperlipidemia, in part, through increased availability of lipoprotein lipases and decreased activities of DGATs,
resulting in reduced lipid accumulation [12, 13]. Obese subjects are reported to be in an inflammation-prone condition [14], which in turn worsens obesity, hyperglycemia, and insulin resistance [15, 16]. With respect to the contribution of obesity to inflammation, it was previously reported that infiltration of macrophages into white adipose tissues was increased in obese status [17], which further increased lipolysis. Macrophage-induced lipolysis subsequently promoted hepatic gluconeogenesis and diminished glucose uptake in skeletal muscle, resulting in insulin resistance and ectopic lipid accumulation in the liver. Of interest, recent studies also demonstrated the direct contribution of adipocytes to the development of inflammatory micromilieu, indicating their involvement in endocrine and/or paracrine modulator functions [18]. Specifically, adipocytes were shown to produce proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, which further modulate both adipocytes and macrophages [14].

Macrophages have evolved to engulf foreign cells and recognize microbe-specific pathogen-associated molecular patterns (PAMPs), including lipopolysaccharides (LPS). The recognition of PAMPs via extracellular and intracellular receptors, such as Toll-like receptors and nucleotide-binding oligomerization domain-like receptors, is further implicated in inflammatory responses for their release of nitric oxide (NO), cytokines, and proinflammatory lipid metabolites such as prostaglandins and leukotrienes. Phagocytic macrophages also trigger adaptive immune responses by expression of antigen-presenting molecules on their surfaces, followed by reciprocal costimulation of T/B-lymphocytes [19, 20].

With advantages of DAGs and MCFAs known, the current study sought to determine the beneficial effects of an edible oil containing MCFA-enriched DAG (MCDG) on adipocytes and macrophages. Conventional canola oil and olive oil served as controls in order to test the substitution by MCDG.

Materials and Methods

MCDG Preparation

MCDG was prepared from canola oil (MSM Milling Company, Australia) and MCFA-triacylglycerols (MCTs) (Ishinwells, Korea). Canola oil mainly consists of TAGs (>96.80%), and its fatty acid composition was determined as oleic acid (C18:1, 60%), linoleic acid (C18:2, 22%), linolenic acid (C18:3, 8%), palmitic acid (C16:0, 5%), and stearic acid (C18:0, 2%). MCT was determined to be composed of caprylic acid (C8:0, 55%) and capric acid (C10:0, 45%). For the production of MCDG, the mixture of canola oil and MCT was hydrolyzed by a lipase in order to liberate glycerols and FFAs. The pool of glycerols and FFAs was re-esterified using a reverse reaction of 1,3-specific immobilized lipase (Novozym 40086; Novozyme, Denmark) [21], followed by distillation for the removal of unesterified FFAs and MAGs. MCDG was further decolorized and deodorized using bleaching earth at 100°C for 1 h and steam at 180°C for 1 h, respectively. The consequent MCDG was determined to be enriched in 1,3-DAG (55%) along with 1,2-DAG (25%) and TAG (20%). For application to the cells, MCDG as well as control oils were dissolved in DMSO (Daejung Chemical, Korea) and further diluted in the culture medium at the working concentration. The final concentration of DMSO in the cell culture medium was maintained at less than 0.01% (v/v), for which toxicity was not observed previously [22].

Cell Culture

The murine macrophage RAW264.7 cell line was purchased from the Korea Cell Line Bank (KCLB, Korea). The cells were seeded on 24-well plates at a concentration of 1.0 × 10^5 cells/ml with Dulbecco’s modified Eagle’s medium (DMEM; Welgene, Korea) supplemented with 10% fetal bovine serum (FBS; Welgene) and 1% antibiotic/antimycotic solution (10,000 U/ml penicillin G, 10,000 µg/ml streptomycin, and 25 µg/ml amphotericin B; Welgene) at 37°C in a 5% CO2 incubator (Model BB15; Thermo Scientific, USA) with or without oil intervention for 24 h. To induce onset of inflammatory responses, macrophages were stimulated with 500 ng/ml of LPS (Sigma-Aldrich Co., USA) for 24 h. The murine preadipocyte 3T3-L1 cell line was purchased form KCLB. Cells were seeded on 24-well plates and maintained in DMEM supplemented with 10% bovine calf serum (Welgene) and 1% antibiotic/antimycotic solution. Cells were fed every 2 days during growth before confluency. Differentiation of confluent preadipocytes (day 0) to adipocytes was induced by 20 mM dexamethasone (Sigma-Aldrich Co.), 0.1 mM 3-isobutyl-1-methlyxanthine (Sigma-Aldrich Co.), and 10 µg/ml insulin (differentiation induction medium; Sigma-Aldrich Co.) in 10% FBS-supplemented DMEM for 2 days. From day 2, the culture medium was replaced every 2 days with DMEM supplemented with 10% FBS and 10 µg/ml insulin (maturation medium) until full differentiation on day 8. Appropriate oil treatments were applied during the whole differentiation period.

Cell Viability Assay

The cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded into 96-well plates at the concentration of 5.0 × 10^4 cells/ml and exposed to MCDG (0.01, 0.1, 0.5, 1.0, 10, and 100 µg/ml) for 24 h. Then, 10 µl of MTT solution (5 mg/ml; Amresco, USA) was added to each well, and the cells were incubated for 4 h at 37°C in a 5% CO2 incubator. After incubation, the supernatant of the cell culture medium was removed, and the MTT formazan crystals were dissolved in DMSO. The absorbance was assessed by a microplate reader (Bio-Rad, USA) at 590 nm to determine the cell viabilities. The results were represented by the percentage of