Anti-inflammatory Effects of Conjugated Linoleic Acid Isomers in Mouse Macrophages, RAW 264.7 Cells

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Abstract

Conjugated linoleic acid (CLA) has anti-inflammatory effects in vitro and in vivo; however the contribution of specific isomers to this effect has not been extensively studied. RAW 264.7 (RAW) cells, mouse macrophage cells, were treated with five different CLA isomers (9*E*,11*E*-, 9*Z*,11*E*-, 9*Z*,11*Z*-, 10*E*,12*Z*-, and 11*Z*,13*E*-CLA) and gene expression microarray was performed and significantly regulated genes by CLA isomers were collected and verified by a real-time PCR. Examination of the biological functions of regulated genes by the five CLA isomers showed distinct properties. Interestingly, 9*E*,11*E*-CLA regulates the largest number of genes in RAW cells and it was the only isomer to increase interleukin 1 receptor antagonist (IL-1Ra). Using PPAR and RXR specific antagonists and coactivator recruitment assays, it was evident that multiple mechanisms were responsible for gene regulation by CLA isomers. 9*E*,11*E*-CLA increased IL-1Ra but decreased of IL-1α, IL-1β and IL-6 at both the mRNA and protein level. The increase of IL-1Ra by 9*E*,11*E*-CLA was transcriptionally regulated and was abolished by an PI3K inhibitor. In conclusion, despite structurally similarity the five CLA isomers show specificity in the alteration of various genes in mouse macrophage cells. The regulation of anti- and pro-inflammatory cytokines by 9*E*,11*E*-CLA in RAW cells demonstrates a potential new therapeutic use of this fatty acids.

Introduction

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (C18:2) discovered as the component of charcoaled meat that inhibits carcinogenesis in animal models of disease [1]. CLA has been extensively studied, both as a nutritional component and as a dietary supplement, for its effects in laboratory animals and humans [2]. The mixture of CLA isomers has been repeatedly demonstrated to possess anti-carcinogenic, anti-atherogenic, anti-inflammatory effects in vitro and in vivo, although some of the effects are not firmly concluded from human clinical studies.

Focus of CLA studies has been exploring not only its effects on various diseases but also effects of individual CLA isomers. Some of the CLA studies showed an isomer-specificity of 9*Z*,11*E*- and 10*E*,12*Z*-CLA. 9*E*,11*E*-CLA was shown to have the strongest anti-carcinogenic effect among 9*Z*,11*E*-, 9*Z*11*Z*-, 10*E*,12*Z*-CLA in human colon cancer [3]. In addition, this particular CLA isomer activates sterol response element binding protein (SREBP) target genes in human macrophages [4]. That is, isomer-specific roles of CLA isomers are crucial in understanding their effects and mechanisms.

We show that gene expression of five CLA isomers (i.e., 9*E*,11*E*-, 9*Z*,11*E*-, 9*Z*,11*Z*-, 10*E*,12*Z*-, and 11*Z*,13*E*-CLA) in mouse macrophages by using high-throughput microarray analysis. The regulation by CLA-isomers is further studied using nuclear receptor antagonists and coactivator recruitment assay. It is hypothesized that the induced IL-1Ra by 9*E*,11*E*-CLA is involved in controlling pro-inflammatory cytokines, which may help explaining how the CLA isomer plays a role to regulate inflammatory genes.

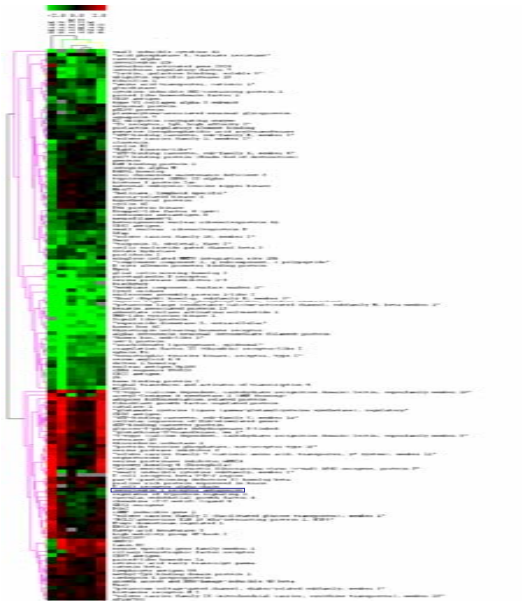

Figure 1. Five CLA isomers regulate gene expression differently in RAW cells. RAW cells were starved for 24 hrs and then treated with 200 μM of CLA isomers for 24 hrs. Total RNA was isolated and was used for microarray. Color scale in the gene tree is from -2.0 (green) to 2.0 (red) that represent decrease and increase respectively using TMeV with Log_2 scale. This tree shows the relevance among CLA isomers and PGJ_2.

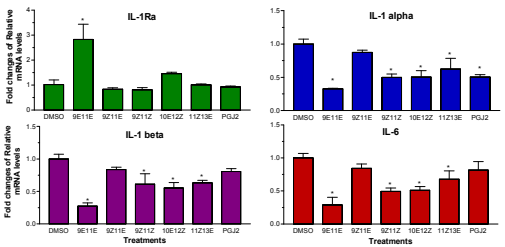

Figure 2. CLA isomers regulates anti-inflammatory cytokine (IL-1 receptor antagonist), and pro-inflammatory cytokines (IL-1α, IL-1β, and IL-6). RAW cells were starved for 24 hrs and then, treated with 200 μM of CLA isomers for 24 hrs with the last 6hrs of 1 μg/mL LPS challenge. Total RNA was isolated and used in RT. Real-time PCR was performed. * indicates a significant difference compared to DMSO group (p<0.05).

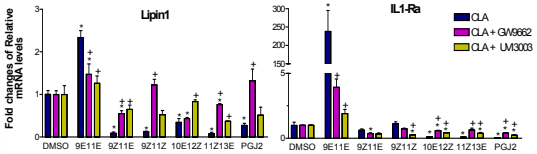

Figure 3. PPAR and/or RXR are involved in regulation of gene expression by CLA isomer in RAW cells. RAW cells were pretreated with pan-antagonist for PPAR and RXR, GW9662 and UVI3003, respectively for 30 mins, and co-treated with 200 μM of CLA isomer for 24 hrs. Total RNA was isolated and used for RT. Real-time PCR was performed. * and + indicate a significant difference compared to each DMSO group and CLA only treated group, respectively (p<0.05).

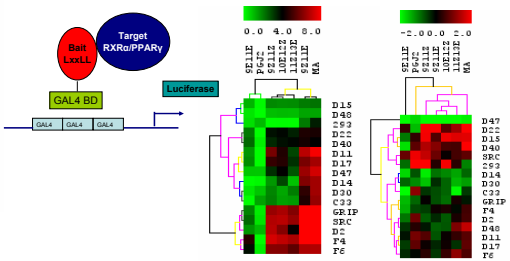

Figure 4. CLA isomers act as selective receptor modulator (SRM), differently recruit coactivators to RXRα and PPARγ. Mammalian 2-hybrid was performed with a pMLXXLL peptide library [5] and pVP16 RXRα and PPARγ LBD by transiently transfecting 3T3-L1 cells. Relative luciferase values were imported to TMeV to view CLA regulation via cluster tree.

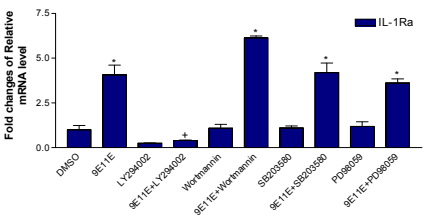

Figure 5. LY294002 abolished the induction of IL-1Ra by 9E,11E-CLA in RAW cells. RAW cells were pretreated with LY294002 (14 μM), Wortmannin (100 nM), SB203580 (1 μM), PD98059 (5 μM) for 30 min and co-treated with 100 μM of 9E,11E-CLA for 10hrs. Total RNA was isolated and used for RT. Real-time PCR was performed for IL-1Ra. * indicates a significant difference compared to DMSO (p<0.05).

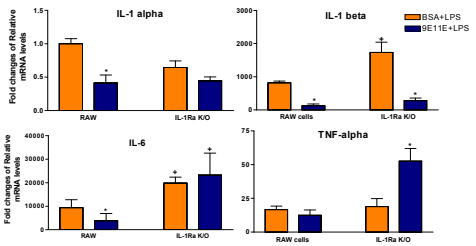

Figure 6. IL-1Ra induction by 9E,11E-CLA decreases pro-inflammatory cytokines. RAW cells were stably transfected with an RNAi for IL-1Ra. Stable cell lines were treated with 100 μM of 9E,11E-CLA for 20 hrs with LPS challenge for the last 6 hrs. Total RNA was isolated and used for RT. Real-time PCR was performed for IL-1α, IL-1β, IL-6, TNF-alpha. * and + indicate a significant difference compared to a BSA+LPS in each cell type and a BSA+LPS in RAW normal cells, respectively. (p<0.05)

Conclusions

- Five CLA isomers regulate gene expression differently in mouse macrophages, RAW 264.7 cells.
- The regulation of various genes by CLA isomers are partially due to PPAR and/or RXR.
- CLA isomers are a selective receptor modulator for PPARγ and RXRα.
- 9*E*,11*E*-, 9*Z*,11*Z*-, 10*E*,12*Z*-, and 11*Z*,13*E*-CLA decrease pro-inflammatory cytokines such as IL-1α, IL-1β, and IL-6.
- 9E,11E-CLA decreases pro-inflammatory cytokines, while increases anti-inflammatory cytokine, IL-1Ra.
- LY294002, but wortmannin, abolishes the induction of IL-1Ra by 9*E*,11*E*-CLA suggesting the regulation is PI3K-independent.

Future Studies

- Mechanistic examination of the IL-1Ra induction by 9*E*,11*E*-CLA
- Examination of the beneficial effects of the 9*E*,11*E*-CLA IL-1Ra induction by examining endothelial cells adhesion assay.

References

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