The 9cis,11trans,13cis Isomer of Conjugated Linoleic Acid Reduces Apolipoprotein B100 Secretion and Triacylglycerol Synthesis in HepG2 Cells

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The physiological effects of 9cis,11trans,13cis-conjugated linoleic acid (9c,11t,13c-CLNA), one of the CLNA isomers, were studied in human hepatoma HepG2 cells. 9c,11t,13c-CLNA significantly decreased apolipoprotein B100 secretion compared with α-linolenic acid (α-LNA). The uptake of 14C-oleate into newly synthesized cellular triacylglycerol was also decreased by 9c,11t,13c-CLNA more than by α-LNA treatment. This is the first study to show the hypolipidemic effect of 9c,11t,13c-CLNA.

Key words: conjugated linoleic acid; apolipoprotein B100; triacylglycerol synthesis; HepG2

Conjugated fatty acid (CFA) is the general term for positional and geometric isomers of polyunsaturated fatty acids with conjugated double bonds. It has been reported that conjugated linoleic acid (CLA), the CFA form of linoleic acid, has favorable physiological effects, such as anti-atherosclerosis, anti-obesity, anti-tumor, and anti-hypertension.1–9) There are also other types of CFA in some plant seed oils. Punicic acid (9cis,11trans,13cis-conjugated linoleic acid; 9c,11t,13c-CLNA) is contained at about 72% in pomegranate seed oil.10) α-Eleostearic acid (9cis,11trans,13trans-CLNA) is contained in bitter gourd oil and tangerine oil at about 60% and 70% respectively.11,12) Catalpa seed oil also contains catalpic acid (9trans,11trans,13cis-CLNA) at about 31% and pot marigold seed oil contains calendic acid (8trans,10trans,12cis-CLNA) at about 33%.13) Although recent studies show that CLNAs, mainly α-eleostearic acid, have some physiological functions, including body fat reduction and anti-tumor activity,10–15) no study has evaluated their effect on apolipoprotein B100 (apoB100) secretion or cellular triacylglycerol (TG) synthesis in the liver.

ApoB100 is an essential component in very-low-density lipoprotein, and its blood level is positively correlated with the incidence of coronary heart disease and atherosclerosis.14,15) Therefore, dietary components that control the rate of apoB100 secretion by the liver are of great interest. Previously we reported that the 10trans,12cis isomer of CLA (10t,12c-CLA) reduces the secretion of apoB100 in HepG2 cells.16) Since more highly unsaturated fatty acids have a stronger physiological effect on lipid metabolism,17) we hypothesized that CLNA also reduces apoB100 secretion in HepG2 cells.

HepG2 cells were purchased from American Type Culture Collection (Rockville, MD, U.S.A.). Purified (99%) 9c,11t,13c-CLNA was prepared by Kaneka (Hyogo, Japan). α-Linolenic acid (α-LNA; 9c,12c,15c-octadecatrienoic acid) and bovine serum albumin (BSA) were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). The experimental medium was 1% BSA–DMEM containing either 9c,11t,13c-CLNA or α-LNA, and fatty acid-BSA complex was prepared as described by Van Harken et al.18) In the present study, cell viability was assessed by 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyl tetrazolium bromide (MTT) cytotoxicity assay,19) and apoB100 secretion was measured by sandwich enzyme-linked immunosorbent assay using rabbit anti-human apoB100 IgG.20) Determination of cellular TG synthesis was carried out as follows: Cells were incubated with experimental medium containing 37 KBq [1-13C] oleate (American Radiolabeled Chemicals, St.Louis, MO, U.S.A.) for 6 h. Total lipids in the cells were extracted and purified by the method of Bligh and Dyer.21) The lipids were fractionated using thin-layer chromatography (TLC) in a solvent mixture of petroleum ether:diethyl ether:acetate (82:18:1, v/v). After separation with TLC, radioactivity in the triacylglycerol fraction was measured with a bio-imaging analyzer (BAS1000, Fuji Photo Film, Kanagawa, Japan).

Human hepatoma HepG2 cells are the most suitable and accessible human-derived cells and retain many of the biochemical functions of human liver parenchymal cells.22) The viabilities of HepG2 cells, as assessed by
References


by the suppression of TG synthesis in HepG2 cells. Hence we consider that the reduction of apoB100 secretion by 9c,11r,13c-CLNA is at least in part attributable to the suppression of cellular TG synthesis in HepG2 cells. In the present study, however, we did not elucidate the underlying mechanisms of the reduction of TG synthesis by 9c,11r,13c-CLNA. Hepatic TG synthesis is regulated by the availability of the fatty acid pool in the liver. Previously we reported that 10r,12c-CLA reduced the hepatic TG level through suppressed activity of fatty acid synthase, a key enzyme in fatty acid synthesis, and enhanced activity of carnitine palmitoyltransferase, a key enzyme in fatty acid β-oxidation, in the liver of obese rats. Therefore, we speculate that 9c,11r,13c-CLNA also suppressed TG synthesis through modulation of these enzyme activities. Further investigation is needed.

In conclusion, we found for the first time that the 9c,11r,13c-CLNA isomer reduces apoB100 secretion perhaps through the suppression of TG synthesis in human liver derived cells. These results might indicate the use of 9c,11r,13c-CLNA for dietary hypolipidemic component.


