Toxicology in Vitro 26 (2012) 985–992

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Human colon cell culture models of different transformation stages to assess conjugated linoleic acid and conjugated linolenic acid metabolism: Challenges and chances

Christian Degen [a,*], Nina Habermann [b], Stefanie Piegholdt [c], Michael Glei [d], Gerhard Jahreis [a]

[a] Department of Nutritional Physiology, Institute of Nutrition, Friedrich-Schiller-University Jena, Dornburger Str. 24, 07743 Jena, Germany
[b] Division of Preventive Oncology, National Center for Tumor Diseases/German Cancer Research Center, Im Neuenheimer Feld 460, 69115 Heidelberg, Germany
[c] Institute of Human Nutrition and Food Science, Christian-Albrechts-University Kiel, Hermann-Rodewald-Str. 6, 24118 Kiel, Germany
[d] Department of Nutritional Toxicology, Institute of Nutrition, Friedrich-Schiller-University Jena, Dornburger Str. 24, 07743 Jena, Germany

ARTICLE INFO

Article history:
Received 27 December 2011
Accepted 3 May 2012
Available online 11 May 2012

Keywords:
Conjugated linoleic acid
Conjugated linolenic acid
Colon adenoma/adenocarcinoma cells
Fatty acid metabolism

ABSTRACT

Both cellular transformation status and cell culture conditions affect fatty acid metabolism. Hence, the incorporation and metabolism of *c*9,*t*11-CLA (conjugated linoleic acid) and other CFAs (conjugated fatty acids) were compared in colon cells (LT-97, adenoma; HT-29, adenocarcinoma). Growth inhibition by CFA in LT-97 cells was assessed via the DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) assay. Basal gene expression of desaturases (Δ5, Δ6 and Δ9) and elongases (1, 2, 5 and 6) was determined in LT-97 using PCR. Analysis of cellular fatty acids revealed a 2-fold higher incorporation of *c*9,*t*11-CLA (40 and 80 μM) in HT-29 cells compared to LT-97 cells. The *β*-oxidized and elongated conjugated dienoic (CD) fatty acids differed by 8-fold (CD-C16:2/CD-C20:2; HT-29: 8:1; LT-97: 1:1). Notably, LT-97 cells were shown to convert conjugated linolenic acid (CLnA) to CLA. Moreover, LT-97 cells revealed no basal expression of *elongase* 2. CLnA caused stronger growth inhibition (⩽80 μM) compared to CLA (200 μM). The results indicate that LT-97 cells represent a superior model to carry out elongation and desaturation studies of unsaturated and conjugated fatty acids compared to HT-29 cells. Nevertheless, further in-depth metabolic and transcriptomic analyses are required to confirm this suggestion.

1. Introduction

An indispensable prerequisite for estimating beneficial but also adverse effects of potential target substances on human health is a precise assessment of the modes and mechanisms of actions of such substances. The method of choice is to conduct (human) *in vivo*-studies. However, the studies often involve costly and laborious procedures, and the methods are sometimes simply impractical or even unethical. Take the case of colon cancer as an example. Here, long-term studies are particularly rare and a follow-up over decades is mostly a demanding procedure for subjects. On the other hand, there are the two large prospective long-term cohorts that are involved in the Design of the Women's Health Initiative clinical trial and observational study (1998) and the European prospective investigation into Cancer cohort (Riboli and Kaaks, 1997) investigating the relationship between diet and different forms of cancer and cardiovascular diseases.

Since collection of human biospecimens is only possible to a limited extent, the method open to scientists for unraveling the interrelation between targets and modes of action mechanistically is to work with *in vitro* cell culture systems. For exploring the changes occurring within the course of colonic malignant transformation, a large set of established adenocarcinoma cell lines of human origin such as HT-29 (Fogh and Trempe, 1975), SW-480 (Leibovitz et al., 1976), CaCo-2 (Fogh et al., 1977), and HCT-116 (Brattain et al., 1981) are available. In contrast, the use of human colon cell lines for examining precancerous stage of tumorigenesis is restricted, as these cells demonstrate finite cell division and growth *per definitionem* and are hence more specific compared to cell cultures of late stage cancer. A cell line representing a "normal

Abbreviations: AA, arachidonic acid; CD, conjugated dienoic; CFA, conjugated fatty acid; CLA, conjugated linoleic acid; CLnA, conjugated linolenic acid; EC_{50}, 50% effective concentration; ELOVL1/2/5/6, fatty acid elongase 1/2/5/6 (elongation of very long chain fatty acids); FA, fatty acid; FADS1, fatty acid desaturase 1 (Δ5-desaturase); FADS2, fatty acid desaturase 2 (Δ6-desaturase); FAME, fatty acid methylesters; FCS, fetal calf serum; HT-29, human colon adenocarcinoma cells; LT-97, human colon adenoma cells; MUFA, monounsaturated fatty acid; PPIA, peptidylprolyl isomerase A (cyclophilin A); PUFA, polyunsaturated fatty acid; SCD, stearoyl-CoA desaturase (Δ9-desaturase); SFA, saturated fatty acid.

* Corresponding author. Address: Department of Nutritional Physiology, Institute of Nutrition, Friedrich-Schiller-University Jena, Dornburger Str. 24, D-07743 Jena, Germany. Tel.: +49 3641 949 616; fax: +49 3641 949 612.

E-mail addresses: b0dech@uni-jena.de, Christian.degen@uni-jena.de (C. Degen).

0887-2333/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tiv.2012.05.002

state" of a colon cell is not available to date and culturing primary colon cells i.e. obtained from human colon biopsies (even only for a few hours) remains a complex process (Scharlau et al., 2009; Wilhelm et al., 2012). However, one of these rare cell lines, the LT-97 is regarded as a model cell line reflecting early carcinogenesis (Richter et al., 2002). Tissue culture with less transformed cells is difficult owing to the diverse requirements necessary to maintain the cell culture including several types of growth medium and different concentrations of supplemented vitamins and trace metals as well as fetal calf serum (FCS). However, results obtained using these cell lines cannot be directly compared to outcomes of related studies using tumor cell lines (Wilhelm et al., 2012). As seen in the literature, disparity in the specifications required for cell culture techniques result in an enormous range of effects for the many different cell lines. A number of studies investigating the effects of food ingredients such as fatty acids (FAs) including conjugated fatty acid (CFA) from lipids (Fauser et al., 2011) on apoptosis induction, cell cycle arrest, growth inhibition in a range of cancer cell types and colon cancer show promising effects.

Two promising CFAs whose anticancer properties are recognized from *in vitro*- as well as *in vivo*-studies include conjugated linoleic acid (CLA) and conjugated linolenic acid (CLnA) (Kelley et al., 2007; Hennessy et al., 2011). CFAs are characterized by possessing diverse positional isomers of different configuration (*all-cis*, *cis*/*trans*, *trans*/*cis*, *all-trans*) and conjugated double bonds (dienoic, trienoic, tetraenoic) (Iwabuchi et al., 2003). CLA is predominantly formed in the paunch of ruminants such as cattle (Kraft et al., 2003), whereas CLnA is found in abundance in bitter gourd seed, snake gourd seed, tung seed, catalpa seed, and pomegranate seed (Özgul-Yücel, 2005; Hennessy et al., 2011).

According to Habermann et al., 2009, the concentration of absorbed polyunsaturated fatty acids (PUFAs) differs in tumor cells compared to precancerous colon cells, HT-29 and LT-97 cells, respectively. Thus, the total quantity of fatty acids (FAs) in the cells also varies (Habermann et al., 2009). In addition, following incubation of the cells with specific FAs, the distribution of FAs is also altered. Moreover, cellular enzymatic patterns in particular impair incorporation and metabolism of the available FAs. In contrast, normal healthy mammalian cells apply an intact enzymatic system (Guillou et al., 2010); however, during colon carcinogenesis enzymes related to FA metabolism underlie transcriptional changes (Kim and Milner, 2011). Thus, enhanced transcription and activity of FASN (fatty acid synthase) and SCD (Δ9-desaturase) (Kelly et al., 1990; Ogino et al., 2008; Furuta et al., 2010) takes place within carcinogenesis and concomitantly, the activity of FADS1 (Δ5-desaturase) and FADS2 (Δ6-desaturase) (Mathers and Bailey, 1975; Meng et al., 2004; Degen et al., 2011) as well as of elongases (e.g. ELOVL2 and 5) is reduced to the degree corresponding to the detection limit (Degen et al., 2011). Hence, knowledge regarding the cellular enzymatic system, especially of desaturases (Δ5, Δ6 and Δ9) and ubiquitary elongases (ELOVL1/2/5 and 6) is necessary to explain differences between CLA and CLnA with respect to both FA metabolism and their effectiveness in e.g. reduction of cell growth in colon cells in different stages of transformation. Additionally, distinct cell culture conditions must also be considered for the evaluation of FA metabolism. Notably, there is a lack of thorough *in vitro*-studies investigating the incorporation and the resulting metabolism of supplemented FAs such as CLA into cellular lipids. Furthermore, the manner in which enzymatic FA metabolism (applied FA vs. metabolites) affects cell growth remains elusive. Consequently, analyses of cellular metabolism require different types of techniques together with an array of methodologies and chromatography procedures especially interfaced with mass spectrometry to determine the cellular transcriptomic and metabolic profiles. However, for characterizing cellular fatty acid metabolism and evaluating the disposition of applied fatty acids after incorporation, the basal expression of major desaturases and elongases in LT-97 cells is crucial. Assuming a dissimilar metabolism in the different cell lines, above all in cell lines of varying tumorigenic origin, we hypothesize that the cellular response to FAs is thereby affected. The aim of this study is to compare the incorporation:

1. of *c*9,*t*11-CLA into cellular lipids of HT-29 and LT-97 cells, and
2. of *t*9,*t*11-CLA as well as *c*9,*t*11,*t*13-CLnA into cellular lipids of LT-97 cells according to standards cell culture conditions, respectively.
3. Furthermore, the impact of the number and geometrical position (*cis*/*trans* vs. *all-trans*) of conjugated double bonds concerned with the growth inhibitory effects of CLA and CLnA was investigated.

2. Material and methods

2.1. Materials

The different CFA isomers (*c*9,*t*11-CLA, *t*9,*t*11-CLA, *c*9,*t*11,*t*13-CLnA, *t*9,*t*11,*t*13-CLnA) were purchased from CPS Chemie (Aachen, Germany), a distributor for Larodan Fine Chemicals (Sweden). Several aliquots of the FA solutions in 100% ethanol were covered with an argon layer and kept at −80 °C until needed for analysis. CPS Chemie (Aachen, Germany), Nu-Chek Prep (Elysian, USA), Sigma (Steinheim, Germany) and Supelco (Taufkirchen, Germany) supplied the FAME (fatty acid methylesters) standards for gas chromatography (GC) analysis. Used cell culture material was obtained from Greiner Bio one (Frickenhausen, Germany) and DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) was received from Carl Roth GmbH (Karlsruhe, Germany). Cell culture media, FCS and supplements were obtained from Biochrom AG (Berlin, Germany), Sigma (Steinheim, Germany), Invitogen (Karlsruhe, Germany) and Merck Biossciences GmbH (Schwalbach, Germany).

2.2. Cell culture

Human colon adenoma cells LT-97 were a kind gift from Professor Marian (Institute for Cancer Research, University of Vienna, Austria) (Richter et al., 2002; Knoll et al., 2006). Human colon adenocarcinoma cells HT-29, obtained from the American Tissue Culture Collection (ATCC) were cultured under standard conditions at 37 °C in a humidified culture incubator with 5% CO_2 and 95% humidity. Both cell lines were grown as monolayers in flasks of T-25 cm^2 and T-75 cm^2 and transferred upon the cell density reaching 70–80% confluence as described previously (Habermann et al., 2009). The culture medium of both cell lines differed enormously (LT-97: 20% L15 (Leibowitz) in MCDB 302 medium, 1 μg/mL hydrocortisone, 10 μg/mL insulin, 5 nM sodium selenite, 0.2 nM trijodo-L-thyronine, 0.4 mM L-glutamine, 50 μg/mL gentamycin, 30 ng/mL epidermal growth factor and 2% FCS; HT-29: DMEM (Dulbecco's Modified Eagle Medium) containing 4.5 g/L glucose, 58 mg/L L-glutamine, without sodium pyruvate and 10% FCS).

2.3. Determination of growth parameter using the DAPI assay

The capability of DAPI to intercalate with the DNA provides a reliable parameter for assessing the cell growth compared to controls and was conducted as described previously (Degen et al., 2011). Cells were treated with up 5–100 μM of CLnA or CLA (10–200 μM) for 24, 48 and 72 h, respectively. Fluorescent intensity of the stable DAPI/DNA complex was measured and the results of DAPI assay were compared to the respective medium controls (<0.2% ethanol) that were set at 100% and expressed as relative values. Data of DAPI assay were calculated as effective concentrations (EC_{50}) that inhibited viability by 50% using the GraphPad PRISM 5