Acyl-CoA thioesterase activity in human placental choriocarcinoma (BeWo) cells: effects of fatty acids

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Abstract

The effects of fatty acids on acyl-CoA thioesterase activity and peroxisome proliferator-activated receptor γ (PPARγ), a regulator of lipid metabolism, were investigated in placental choriocarcinoma (BeWo) cells. Substrate preference for acyl-CoA thioesterase was in the following order; γ-linolenoyl-CoA ≫ arachidonoyl-CoA ≫ palmitoyl-CoA ≫ linoleoyl-CoA. However, when these cells were incubated with fatty acids, acyl-CoA thioesterase activity was increased by both conjugated linoleic and γ linolenic acids, but not by docosahexaenoic and eicosapentaenoic acids. In addition, these fatty acids also increased expression of PPARγ in these cells, suggesting a putative relationship between free fatty acid generated by acyl-CoA thioesterase and expression of PPARγ. Since expression of PPARγ is critical for feto-placental growth, these fatty acids may be important during pregnancy.

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1. Introduction

Delivery of fatty acids from the maternal circulation to the fetus occurs via the placenta, which acts as a physical barrier between the maternal and fetal blood. Essential fatty acids (EFAs) and their long-chain polyunsaturated fatty acids (LCPUFAs) derivatives are very important for the development of the fetus [1,2]. They play a crucial role in the structure and function of membranes, with LCPUFA being especially important to fetal brain and retina development [2,3]. Although the mechanism involved in the transfer of these fatty acids across the placenta is now largely known [1,3], very little information is available on the fatty acid metabolism during their transit across the placenta syncytiotrophoblasts. Critical regulators in cellular lipid homeostasis are lipid-sensing nuclear transcription factors known as peroxisomal proliferator-activated receptors (PPARs) [4]. There are several types of PPAR such as PPARα, PPARβ (also called δ or NUC1) and PPARγ (PPARγ-1 and PPARγ-2) [4–6]. PPARγ found in adipose tissue as well as in placenta [6,7] is thought to be involved in the stimulation of lipid accumulation by controlling expression of several lipid metabolic genes such as fatty acid transporter/bindng proteins, lipase, the acyl-CoA synthetase, etc. [8,9]. PPARs are also involved in the regulation of cellular proliferation, differentiation and apoptosis [4–6]. PPARγ gene knockout in mice results in severe placental and fetal abnormalities [7]. Recent studies have demonstrated that PPARγ is responsible for the synthesis of hCG, a hormone essential for human pregnancy, and also human placental lactogen, human placental growth hormone, and leptin secretion [10]. Despite its critical importance in feto-placental development, the natural ligands for PPARγ are still not known. PPARs are transcriptionally activated by fatty acids (or other ligands, hypolipidamic agents such as thiazolidinediones, prostaglandins, and eicosanoids) and then form a heterodimer with the retinoid X receptor (RXR) [8]. LCPUFAs and their derivatives which may serve as potential natural ligands for PPARγ can be obtained from the maternal circulation by the placenta via the fatty acid transport system [1,3]; however, their role as natural ligands are yet to be confirmed. Besides being potential ligand, fatty acids may also modulate PPARγ expression [8,11,12]. Fatty acids and fatty acid derivatives, in particular, products of the cyclooxygenase and lipoxygenase pathways, are known to activate members
of the PPAR family whereas the convincing evidence still remains to be shown that CoA esters can act on PPARs [13]. PPARγ binds free fatty acids (FFAs) not its fatty acyl-CoA; therefore, it is important to investigate how the levels of FFAs are regulated in the cells. Acyl-CoA thioesters cleave acyl-CoA to produce FFA and CoASH. They exist in various tissues of many organisms and are widely distributed in the cell (rat liver mitochondria, peroxisomes, cytosol and microsomes [14,15], guinea-pig brown adipose tissue mitochondria [16], Candida rugosa [17], lactating-rabbit mammary gland cytosol [18].

The acyl-CoA thioesterase activity of cellular compartments is the result of several enzymes [16,17]. There are three types of acyl-CoA thioesterases, according to their substrate specificity: (i) short-chain acyl-CoA thioesterases (acytetyl-CoA hydrolases, EC. 3.1.2.1; short-chain acyl-CoA thioesterase, EC. 3.1.2.3–5 and EC. 3.1.2.11 [19]); (ii) medium-chain acyl-CoA thioesterases [20]; (iii) long-chain acyl-CoA thioesterases (palmitoyl-CoA hydrolases, EC. 3.1.2.2 [14]). Several lines of evidence suggest that PPARs could be involved in the regulation of the expression of these enzymes [12,16,21]. So far, very little information is available on acyl-CoA thioesterase in human placental trophoblast cells. Nevertheless, the transcript of an adrenocorticotropin-regulated phosphoprotein and intermediary in steroids synthesis, which is similar to an acyl-CoA thioesterase, has been detected in placenta [22].

Therefore, the main aims of the present study were to investigate the presence of acyl-CoA thioesterase in the placental choriocarcinoma (BeWo) cells, and their expression by several dietary fatty acids. In addition, we investigated expression of PPARγ by different fatty acids in BeWo cells. BeWo cells are a good experimental model for placental trophoblasts to study lipid metabolism as these cells express almost all lipid metabolic genes [23].

In the present paper, we report the presence of acyl-CoA thioesterase activity in placental cells and their regulation by different dietary fatty acids. Our data suggest that dietary LCPUFAs, specially γ-linolenic acid (GLA) may have important role in the growth and development of feto-placental unit.

2. Materials and methods

2.1. Materials

- 15-deoxy-Δ12,14-prostaglandin J2 (15-deoxyPGJ2) was obtained from Biomol, USA. 3-(4,5-dimethyl thiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), γ-linoleoyl-CoA, arachidonoyl-CoA, linoleoyl-CoA, palmitoyl-CoA and acetyl-CoA were obtained from Sigma, Poole, UK. Proetase inhibitor cocktail set III was obtained from Novo biochem, UK. Trypsin-EDTA solution, penicillin–streptomycin solution, Nutrient Ham’s Mix-F-12 with glutamax-I and William’s medium E with glutamax-I were obtained from Gibco Life Technologies, Scotland. BeWo cells were from the European Collection of Animal Cell Cultures (Salisbury, UK).

Polyconal antibody (goat) raised against PPARγ was from Santa Cruz Biotechnology, USA. Horse radish peroxidase (HRP) linked anti-sheep/goat IgG was from Scottish antibody production unit, Carluke. Other reagents used were of the highest purity available from Sigma Chemical Co., Poole, UK.

2.2. Cell culture

BeWo cells were obtained from European Collection of Animal cell cultures and were grown in Ham’s F12 medium containing 10% fetal bovine serum (FBS), 6 mM glucose and 100 U/ml penicillin-streptomycin. Cells were maintained as monolayers in 25 cm² tissue culture flasks at 37°C with a 5% CO₂-balanced air and 100% humidity atmosphere [24]. At confluence, they were subcultured using a Trypsin–EDTA solution to suspend the cells. Media were renewed three times weekly. Cell viability was routinely tested as the ability to exclude trypan blue. For experiments, cells from early confluent monolayers were dispersed and replated in 58 mm × 115 mm culture dishes. When the cells reached 90–95% confluence, the monolayer was detached from the flask bottom and either subcultured into new flask or seeded onto 100 mm² culture dishes for experimentation.

2.3. Preparation of fatty acids

The preparation of the fatty acids was done according to the method described by Campbell et al. [24]. The appropriate amount of fatty acid was added to this BSA culture medium to obtain a 25 μM incubation media, with a fatty acid/albumin ratio of 1:3. The BeWo cells were grown to a level of confluence between 90% and 95% confluence in the cell culture dishes. Prior to the addition of the incubation medium, cell monolayers were incubated in 1% FBS culture medium for 2 h at 37°C. Cells were incubated for 16 h at 37°C in the presence and absence of fatty acids. On removal from the incubator, the monolayers were first rinsed in a BSA–PBS solution and then ice-cold PBS. The monolayer was then scraped from each dish into 900 μl of the cell-removal buffer and transferred into a 1.5 ml microcentrifuge tube. The cells were then homogenised in PBS containing 1% protease inhibitor cocktail. The cell homogenate was then used either for acyl-CoA thioesterase assay or Western Blot analysis for PPARγ.
2.4. Assay of acyl–CoA thioesterase activity

Acyl-CoA thioesterase activity was routinely followed spectrophotometrically by measuring the appearance of the free thiol groups of CoASH with DTNB, as described earlier [19]. The reaction mixture contained 50 mM KCl, 10 mM Hepes (pH 7.4) and 0.1 mM DTNB. The appropriate acyl-CoA was preincubated and the reaction was started by the addition of enzyme. The reaction was followed at 412 nm using Unicam 8700 UV/VIS spectrophotometer. The amount of thiol groups was calculated from molar extinction coefficient \( E = 13\,6001/mol/cm \). Protein was determined according to Bradford method [25] using BSA as a standard.

2.5. Western Blot analysis of PPARγ of BeWo cells

Monospecific polyclonal antisera raised against the human PPARγ was used. Western Blot analysis of BeWo cell homogenates were carried out as described before [23,24]. Polyacrylamide gel electrophoresis of cellular proteins in the presence of SDS was carried out under reducing conditions on SDS–PAGE gel homogenous 20 (Phast system, Pharmacia, UK). After electrophoresis, proteins were transferred onto a nitrocellulose membrane by diffusion blotting at 70°C for 1 h. The membranes were probed for the presence of PPARγ by incubating with polyclonal antiserum to this protein. Antibody–antigen complexes were then detected with HRP-anti-IgG fraction of donkey polyclonal antiserum (Scottish Antibody Production Unit).

2.6. Cell death analysis

Mitochondrial variability, as an index of cell viability, was measured using the MTT method [26]. BeWo cells were grown in a 96-well microtitre plate until 90–95% confluent. The cells were then incubated with fatty acids and 15-deoxyPGJ2. After the 16-h incubation period the medium was removed and replaced with 100 µl of fresh serum-free culture medium. Ten microlitres of the stock MTT solution was then added to each well. A negative control of 10 µl MTT solution and culture medium was also included. The plate was then incubated further for 4 h at 37°C. At the end of this incubation period, 100 µl of the SDS solution was added to each well and mixed thoroughly with the pipette. The microplate was then incubated overnight at 37°C. The next day each sample was again mixed with MTT reagent and the absorbance was read at 570 nm.

2.7. Statistical analysis

Data are presented as mean ± SEM. Statistical significance was determined using a two-tailed Student’s t-test; \( P \) values were considered significant when equal to or less than 0.05.

3. Results

3.1. Acyl-CoA thioesterase activity in BeWo cells

Acyl-CoA thioesterase activity of BeWo cell extract was measured spectrophotometrically, following the appearance of CoASH. Acyl-CoA thioesterase activity was measured using different substrates with different chain lengths such as arachidonoyl-CoA, γ-linoleoyl-CoA, γ-linoleoyl-CoA, palmitoyl-CoA and acetyl-CoA (Fig. 1). Acyl-CoA thioesterase activity of BeWo cell was mainly specific to γ-linoleoyl-CoA and arachidonoyl-CoA. At 30 µM followed by substrate concentration, 65 ± 9 nmol CoASH/min/mg was released when γ-linoleoyl-CoA was used, followed by arachidonoyl-CoA (49 ± 6 nmol CoASH/min/mg of protein) and palmitoyl-CoA (31 ± 8 nmol HSCoA/min/mg of protein) and linoleoyl-CoA (27 ± 5 nmol HSCoA/min/mg of protein). No short-chain acyl-CoA thioesterase activity was observed in BeWo cells. No short chain acyl-CoA thioesterase activity was observed in BeWo cells. Acyl-CoA thioesterase activity was also measured in a fraction containing only heavy mitochondria, solubilised with Triton X-100. Different concentrations of linoleoyl-CoA were tested (data not shown). The measured activity was very low, and a rate was observed only after the first minute of incubation. Nevertheless, a very low level of acyl-CoA thioesterase activity exists in heavy mitochondria.

3.2. Effects of fatty acids on Acyl-CoA thioesterase activity

Effect of incubation of BeWo cells with fatty acid solutions on expression of acyl-CoA thioesterase activity was then investigated. Acyl-CoA thioesterase activity was measured after incubation of BeWo cells with fatty acid bound to BSA solutions (oleic acid (OA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA), conjugated linoleic acid (CLA), GLA, and albumin alone as a control). Hydrolysis rates were significantly different, depending on the fatty acid present in the incubation medium. EPA and DHA and OA had no effect on acylCoA thioesterase activity compared with controls. But both CLA and GLA increased acylCoA thioesterase activity (25 ± 3.4 CoASH nmol/min/mg protein and 28 ± 4.6 CoASH nm/min/mg of protein, respectively) compared with control (17 ± 4.3 nmol CoASH/min/mg of protein, \( P < 0.05 \) (Fig. 2). AA also increased the activity but did not reach the significance (\( P > 0.05 \)).
3.3. Effects of fatty acids on PPARγ expression in BeWo cells

The effect of incubation with fatty acid bound to BSA (GLA, CLA, DHA, AA, linoleic acid (LA) and BSA alone as a control), on PPARγ expression was investigated by Western Blotting, using anti-PPARγ antibody, which reacts with both PPARγ1 and PPARγ2. Fig. 3 shows the expression of PPARγ expression in these cells incubated with different fatty acids. Although all LCPUFAs used in this experiment increased the expression of PPARγ in these cells compared with control, CLA and GLA were much stronger inducers of PPARγ expression (Fig. 3).

3.4. Effects of fatty acids and other PPARγ ligands on cell death

MTT assay (a measure of mitochondrial activity) was performed to monitor cell death in the presence of fatty acids and 15-deoxyPGJ2, a ligand for PPARγ [27]. Concentration response study with the compounds above revealed that only 15-deoxyPGJ2 was capable of killing the cells after 16 h incubation. The reduction in MTT activity in response to 15-deoxyPGJ2 was 50% at 10 μM, whereas only modest loss (20%) of MTT activity was observed in the case of other fatty acids (GLA, EPA, DHA, AA, and PA (up to 20 μM)).

4. Discussion

Long-chain fatty acyl-CoA esters are important intermediates in degradation and synthesis of fatty acids, as well as in having important function in regulation of intermediary metabolism and gene expression. Although the physiological functions for most
Expression of PPARγ in BeWo cells.

Fig. 3. Effect of incubation of fatty acids on PPARγ expression in BeWo cell. BeWo cells were incubated for 6 h with 25 μM fatty acid bound to albumin (FA:BSA ratio of 1:3) or with BSA alone. Gel obtained by electrophoresis SDS-PAGE, and stained with Coomassie brilliant blue. The gel was run with 20 μg of proteins. Westerns Blots obtained using anti-PPARγ antibodies (dilution 1/500). Proteins were transferred from gel to PVDF membrane (oleic acid, OA; γ-linolenic acid, GLA; docosahexaenoic acid, DHA, conjugated linoleic acid, CLA).

Acyl-CoA thioesterases have not yet been elucidated, several data suggest that these enzymes may involve in lipid metabolism by modulation of cellular concentration of acyl-CoA and FFA. In this paper, we report that placental trophoblast cell line (BeWo) expresses acyl-CoA thioesterase activity. Substrate preference for acyl-CoA thioesterase was in the following order: γ-linolenoyl-CoA ≥ arachidonoyl-CoA > palmitoyl-CoA > linoleoyl-CoA. This indicates that these acyl-CoA derivatives of LCPUFA are preferentially hydrolysed to produce their respective FFA. It has been shown that acyl-CoA thioesterase hydrolysed preferentially arachidonoyl-CoA to AA in the cytosol of rabbit kidney medulla [28]. Thus, this enzyme can supply AA for prostaglandin synthesis in this region. Therefore, thioesterase activity in these cells may play specific important role by supplying LCPUFA as FFA for cellular metabolism. In fact, the fate of fatty acids in cellular metabolism depends on several physiological or pathological factors. Under normal conditions, fatty acids are mainly beta-oxidised in mitochondria and peroxisomes or incorporated into triacylglycerols and phospholipids. A prerequisite for these reactions to occur is the activation of the fatty acids to their corresponding CoA-thioesters by acyl-CoA synthetases which are present in several cellular compartments [29–31]. Long-chain acyl-CoA thioesterase, in theory a counteracting enzyme activity, has also been described in several cellular compartments [30,31]. The regulation of the thioesterase activity by treatment of rats with peroxisome proliferators has been studied in mitochondria, peroxisomes, microsomes and cytosol. Although only a few long-chain acyl-CoA thioesterases have been isolated and characterised, the strong induction of some of these enzymes by peroxisome proliferators suggests that their functions may be related to lipid metabolism [16,21,32].

In this study, we also demonstrate that certain fatty acids, such as GLA also up-regulated acyl-CoA thioesterase activity in placental cells, possibly through the increased expression of PPARγ. In these cells, 15-deoxyPGJ2 induced apoptosis as determined by MTT assay, whereas GLA and other fatty acid although increased PPARγ expression, it had minimal effects. While 15-deoxyPGJ2 has been linked with the induction of cell cycle arrest and apoptosis, the ligand functions and preference of the PPARγ are relatively distinct. Recently, it has been shown that PPARγ modulate human trophoblast function in a ligand-specific manner [33]. Exposure of cultured primary human trophoblasts to troglitazone enhances biochemical and morphological trophoblast differentiation, and hCG synthesis, whereas 15-deoxyPGJ2 diminishes trophoblast differentiation. Furthermore, 15-deoxyPGJ2 up-regulates p53 expression and promotes apoptosis [33], as we observed in BeWo cells. Since 15-deoxyPGJ2, the putative natural PPARγ ligand is responsible for placental apoptosis, this raises the possibility of existence of other hitherto unknown natural ligands that are responsible for placental growth and differentiation. Derivatives of GLA and other LCPUFA may act as such type of ligands. Our observation may have profound implications in human placental biology. Dysfunction of the human placenta may result in fetal growth restriction, a condition associated with abnormal trophoblast differentiation, and enhanced apoptosis may be triggered by 15-deoxyPGJ2. In these conditions, inhibition of 15-deoxyPGJ2 production and/or supplementation of GLA and other LCPUFAs might reverse the apoptotic pathway and support placental growth and development. These fatty acids may thus also allow placental development by increasing PPARγ levels which in turn increases expression of several lipid metabolic genes such as FAT, FATP, FABPpm, FABPs, hCG and other placental hormones.

In conclusion, placental trophoblast cells express acyl-CoA thioesterase activity which prefers LCPUFA, GLA and AA. Incubation of placental cells with GLA and AA increased acyl-CoA thioesterase activity and PPARγ expression, but had very little effect on cell death. These fatty acids may thus play a critical role in fetoplacental growth and development, as PPARγ is critically associated with fetal and placental growth and development.
References


