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Enhanced oleate uptake and lipotoxicity associated with laurate

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ABSTRACT

Free fatty acids have been reported to induce cell death (lipotoxicity), but the effects depend on the carbon chain length and number of double bonds. Medium-chain saturated fatty acids (MC-SFAs), such as laurate, have less lipotoxicity than long-chain saturated fatty acids (LC-SFAs), such as palmitate. Monounsaturated fatty acids, such as oleate, have also been reported not only to exert cytotoxic effects, but also to reduce the lipotoxicity of LC-SFA. However the interaction between MC-SFA and oleate with respect to cell death is unclear. In this report, we found that lipotoxicity was enhanced by a combination of laurate and oleate relative to either fatty acid alone. The possible mechanisms involved were examined by measuring the production of reactive oxygen species, mitochondrial depolarization, caspase-3 activity, and lipid droplet formation. Although the stress signals and cell death pathways were distinct among different cell types, we found a common phenomenon of enhanced lipid droplet formation in all cells tested. Using fluorescent- or radioisotope-labeled fatty acids, we found that oleate, but not laurate, increased the uptake of fluorescent-labeled fatty acids, and the combinatory effect was more efficient than with oleate alone. We also found that laurate increased oleate uptake, but the effect of oleate on laurate uptake varied among cell types. These results suggest that laurate enhances the influx rate of oleate, the increased intracellular concentration of which not only enhances lipid storage, but also induces cell death by lipotoxic stress responses, which vary according to cell type.

1. Introduction

Saturated fatty acids (SFAs) represent endogenously synthesized and dietary compounds that substantially contribute to tissue building and serve as an energy source as well as facilitating the development of diseases [1–3]. In mammalian cells, SFAs vary in their carbon chain lengths from 2 to 24 carbons, and the different chain lengths impart various physiological activities to the fatty acids in many cell types [1–3]. Current evidence suggests that an accumulation of long-chain (LC)-SFAs (more than 14 carbons) leads to lipid-mediated cell dysfunction [1–3]. Many investigators have also demonstrated that LC-SFAs, especially palmitate and stearate, induce apoptosis within a variety of cell types [4–14]. LC-SFAs must be activated to long-chain acyl-CoA for oxidation or use in cellular processes such as membrane formation and lipid storage. Many studies have reported that LC-SFAs induce cell death by triggering production of reactive oxygen species (ROS) [15], causing dysfunction of membrane fluidity by changing the fatty acid content [4,16], and inducing stress signals [5–8,20].

On the other hand, long chain monounsaturated fatty acids (MUFAs), such as oleate and palmitoleate, have lower cytotoxicity than LC-SFAs, but a combination results in an even lower cytotoxic effect, suggesting that MUFAs can inhibit LC-SFA cytotoxicity [4,9,17–20,37]. Following treatment of cells with palmitate and oleate, the intracellular membrane composition of oleate increases to maintain high fluidity and suppress stress signals induced by the treatment with palmitate alone [21,22]. Thus, it appears that MUFAs can promote detoxification of LC-SFAs by altering their partitioning within cells.

In contrast to LC-SFAs, medium chain-saturated fatty acids (MC-SFAs) such as caprylate (8 carbons), caproate (10 carbons), and laurate (12 carbons), are thought to be less cytotoxic than LC-SFAs [23]. MC-SFAs have physical and metabolic properties that are distinct from those of LC-SFAs, which make them a readily available cellular energy source. Few papers have studied cell death induction by MC-SFAs [24], and any interaction between MC-SFA and oleate in the context of cell death has not yet been evaluated precisely. In this report, we found that co-stimulation

Abbreviations: LC-SFAs, long-chain saturated fatty acids; MC-SFAs, medium-chain saturated fatty acids; ROS, reactive oxygen species; SFAs, saturated fatty acids; TG, triacylglycerol

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of laurate with oleate promoted more cytotoxic activity than each alone in several cell types. We also showed that the combination of fatty acids enhanced the formation of lipid droplets and increased fatty acid uptake, especially laurate, which greatly enhanced oleate uptake. Our results highlight the ability of laurate to increase fatty acid uptake and decrease the threshold of oleate cytotoxicity.

2. Materials and methods

2.1. Cell culture

Cells were obtained from the RIKEN Cell Bank (Tsukuba, Japan) and the Health Science Research Resources Bank (HSRRB) (Osaka, Japan) and maintained at 37 °C in a humidified 5% (v/v) CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 10 U/ml penicillin, and 10 g/ml streptomycin, as described previously [25,26].

2.2. Fatty acid treatment

Fatty acids were obtained from Wako (Osaka, Japan), Sigma (St. Louis, MO), and Tokyo Chemical Industry Co., Ltd. (TCI, Tokyo, Japan). Stock fatty acid solutions (100 mM) in dimethyl sulfoxide (DMSO) were diluted in medium containing 0.5% (w/v) fatty acid-free bovine serum albumin. Cells were seeded at 2×10^5/well into 96-well plates. After 24 h of culture, fatty acids (0.5–1 mM) were added to the media. All experiments were carried out at 37 °C under a humidified 5% (v/v) CO_2 atmosphere.

2.3. MTT assay

The MTT assay was used to determine cell viability; the assay is based on bioreduction of the tetrazolium compound MTT [3-(4,5-dimethylthiazolyl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] and the electronic coupling reagent phenazine ethosulfate (PES) to yield a colored formazan product, the absorbance of which is proportional to the number of viable cells. Briefly, after fatty acid treatment for 24–48 h, MTT solution was added to media, the plates were incubated at 37 °C for 2–4 h, and the absorbance of each supernatant was measured at 535 nm, according to the manufacturer's instructions.

2.4. Flow cytometry

Cells were treated with fatty acids for 1–6 h and stained with NucView™ 488 (Biotium, Hayward, CA) to determine the extent of caspase-3 activation; with tetramethylrhodamine methylester (TMRM, Invitrogen) to assess the integrity of the mitochondrial membrane; or with 2′,7′dichlorofluorescin diacetate (DCFDA, Invitrogen) to detect reactive oxygen species (ROS), according to the respective manufacturers' instructions. Fluorescent cells were detected using a JSAN platform (Bay Bioscience, Kobe, Japan) and cell populations were analyzed with the aid of Flowjo software (TreeStar, Ashland, OR).

2.5. Measurement of fatty acid uptake

Cells were incubated with a fluorescent fatty acid analog, C1-BODIPY 500/510 dodecanoic acid (Molecular Probes, Eugene, OR), or radioisotope-labeled fatty acids, thus [1-^{14}C]-oleate, laurate, or palmitate (PerkinElmer, Waltham, MA), with or without addition of other fatty acids, for 1–4 h [27]. Then the cells were washed three times with PBS, lysed in NaOH, and the suspensions were neutralized with acetic acid. Then the cell lysates were transferred to either 96-well black microwell-plates or scintillation vials containing Sintisol (Nacarai Tesque, Kyoto, Japan). Fluorescence intensities and radioactivity levels were measured using a Fluoroscan Ascent platform (Thermo-Scientific, Rochester, MA) or a liquid scintillation counter, respectively.

3. Results and discussion

Previous reports have shown that palmitate, an LC-SFA, induced cell death in several cell types but that its cytotoxicity was ameliorated when combined with oleate [2,3]. Although the MC-SFAs (caprylate, caproate, and laurate) were less cytotoxic than palmitate, any combinatory effect with oleate on cytotoxicity remains unknown. To test the combinatory effect of oleate with MC-SFAs on cell viability, we stimulated HT1080 cells, a human fibrosarcoma cell line, using MC-SFAs with or without oleate. When the cells were stimulated with each fatty acid alone, palmitate was more cytotoxic than laurate and oleate, but these fatty acids still exhibited cytotoxic effects at higher concentrations (Fig. 1). As shown in previous studies, co-stimulation by palmitate (0.5 mM) with oleate (1 mM) caused much less lipotoxicity (more than 80% viability) than that by palmitate alone; however, a mixture of MC-SFA and oleate effectively induced cell death, especially in the case of laurate (less than 10% viability; Fig. 2a and b).

To investigate whether the combination of laurate and oleate exerts the same effects in other cell lines, we tested the combination on 10 additional human and mouse cancer cell lines (A549, Caco2, Ehrlich, HeLa, HepG2, Jurkat, Neuro2a) and cell lines derived from normal tissues (JB6, MRC-5, NIH-3T3). The combination induced a variable but appreciable degree of cell death in all cell lines tested (Fig. 3). These results suggest that the effect of palmitate is different from that of laurate when mixed with oleate.

Long-chain saturated fatty acids have been shown in several cell types to produce reactive oxygen species (ROS), decrease mitochondrial membrane potential, and induce caspase-3 activation, which are hallmark features of LC-SFA-induced cell death [2,3,15]. To assess the effects of a combination of laurate and oleate on ROS production, HT1080, HepG2, and Jurkat cells were treated with laurate in combination with oleate for 1 h. As shown in Fig. 4, ROS levels were increased to a greater extent by exposure of Jurkat cells to the combination (6-fold); however, the effects were less in HT1080 and HepG2 cells (less than 3-fold) than Jurkat cells. The caspase-3 activation induced by laurate with oleate occurred at 6 h in Jurkat cells (more than 60%), but not in HT1080 or HepG2 cells (less than 20%, Fig. 5). The loss of mitochondrial membrane potential is one of the steps preceding caspase activation during apoptosis. As shown in Fig. 6, the combination of laurate and oleate induced a significant increase in the percentage of Jurkat cells with low mitochondrial membrane potential (more than 60%) at 6 h; however, the treatment did not induce depolarization of the membrane potential in HT1080 and HepG2 cells (less than 10%), suggesting that apoptotic processes such as ROS production, caspase-3 activation, and decreased mitochondrial membrane potential induced by the combination of laurate and oleate occur in a cell-type-dependent manner.

It has been shown that triacylglycerol (TG) synthesis and the formation of lipid droplets are stimulated by oleate as well as laurate in tissue culture cells [28–31]. Therefore, we investigated the effect of the combination of fatty acids on lipid droplet formation using a fluorescent indicator. Lipid droplet formation was increased by treatment with either laurate or oleate alone; however co-treatment with laurate and oleate dramatically enhanced lipid formation in all cell types (Fig. 7).

Next, we examined the effect of fatty acid uptake by each fatty acid alone or by a mixture of laurate and oleate. Although the