EBioMedicine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Trans-Fats Inhibit Autophagy Induced by Saturated Fatty Acids

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ARTICLE INFO

Article history:
Received 1 November 2017
Received in revised form 23 March 2018
Accepted 23 March 2018
Available online xxxx

Keywords:
Fasting
Ketogenic diet
Immune response
Immunosurveillance
Obesity
Aging
Cytoprotection
Systems biology

ABSTRACT

Depending on the length of their carbon backbone and their saturation status, natural fatty acids have rather distinct biological effects. Thus, longevity of model organisms is increased by extra supply of the most abundant natural *cis*-unsaturated fatty acid, oleic acid, but not by that of the most abundant saturated fatty acid, palmitic acid. Here, we systematically compared the capacity of different saturated, *cis*-unsaturated and alien (industrial or ruminant) *trans*-unsaturated fatty acids to provoke cellular stress *in vitro*, on cultured human cells expressing a battery of distinct biosensors that detect signs of autophagy, Golgi stress and the unfolded protein response. In contrast to *cis*-unsaturated fatty acids, *trans*-unsaturated fatty acids failed to stimulate signs of autophagy including the formation of GFP-LC3B-positive puncta, production of phosphatidylinositol-3-phosphate, and activation of the transcription factor TFEB. When combined effects were assessed, several *trans*-unsaturated fatty acids including elaidic acid (the *trans*-isomer of oleate), linoelaidic acid, *trans*-vaccenic acid and palmitelaidic acid, were highly efficient in suppressing autophagy and endoplasmic reticulum stress induced by palmitic, but not by oleic acid. Elaidic acid also inhibited autophagy induction by palmitic acid *in vivo*, in mouse livers and hearts. We conclude that the well-established, though mechanistically enigmatic toxicity of *trans*-unsaturated fatty acids may reside in their capacity to abolish cytoprotective stress responses induced by saturated fatty acids.

1. Introduction

Autophagy is a phylogenetically conserved cellular stress response in which portions of the cytoplasm are sequestered in two-membraned autophagosomes that fuse with lysosomes for bulk degradation of their luminal content (Morel et al., 2017; Bento et al., 2016). Autophagy is induced by various stimuli including nutrient shortage (that triggers the digestion of macromolecules to generate energy), adaptation to changing environmental conditions (that may induce the destruction of portions of the cell to rebuild new organelles), as well as sublethal damage (that requires the recycling of dysfunctional organelles and repair responses to increase cellular fitness) (Kaur and Debnath, 2015). As a result, periodic or chronic stimulation of autophagy acts as a cytoplasmic rejuvenation mechanism that increases the longevity of model organisms including yeast, nematodes, flies and mice (Melendez et al., 2003; Tavernarakis et al., 2008; Rubinsztein et al., 2011; Pyo et al., 2013; Green and Levine, 2014; Eisenberg et al., 2016; Ho et al., 2017). Several clinically relevant syndromes leading to premature neurodegeneration and general aging are linked to autophagy defects, (Menzies et al., 2015; Lopez-Otin et al., 2016) suggesting that autophagy may play a general health-improving role in humans as well.

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https://doi.org/10.1016/j.ebiom.2018.03.028

Please cite this article as: Sauvat, A., et al., Trans-Fats Inhibit Autophagy Induced by Saturated Fatty Acids, EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.03.028

Recently, the impact of fatty acids (FAs) on autophagy regulation has attracted some attention. The most abundant natural FAs present in the human body and food are the saturated FA palmitic acid (PA) and the mono-*cis*-unsaturated oleic acid (OL). Strikingly, these two FAs have rather distinct cellular effects in the sense that PA causes the activation of the diabetogenic stress kinase Jun N-terminal kinase-1 (JNK1), while OL rather inhibits PA-induced JNK1 activation (Holzer et al., 2011). Moreover, PA stimulates a canonical pathway of autophagy that, downstream of JNK1, depends on the enzymatic activity of the Beclin 1 (BCLN1)/phosphatidylinositol 3-kinase, catalytic subunit type 3 (PIK3C3) complex generating phosphatidylinositol (3)-phosphate (PI3P), while OL elicits the JNK1 and BCLN1/PIK3C3-independent association of the autophagosome-linked microtubule-associated protein 1A/1B light chain 3 (MAP1LC3, best known as LC3) with the Golgi apparatus (Niso-Santano et al., 2015b; Bankaitis, 2015; Niso-Santano et al., 2015a). This difference in pro-autophagic signals elicited by PA and OL is evolutionarily conserved in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, mice and cultured human cells (Niso-Santano et al., 2015b; Enot et al., 2015). Moreover, it extends to other saturated FAs (that behave like PA) and mono- or poly-*cis*-unsaturated FAs (that behave like OL) with respect to JNK1 and PIK3C3 activation (Niso-Santano et al., 2015b).

Unsaturated FAs that are contained in vegetables and human tissues are *cis*-isomers, meaning that the unsaturation causes a kink in the FA chain (Duplus et al., 2000). *Trans*-unsaturated FAs, which lack such a kink, meaning that their physicochemical properties are rather different, are generated during industrial food processing, for example during partial hydrogenation of fats resulting in the isomerization of *cis*-unsaturated FAs into their *trans*-unsaturated isomers (Tzeng and Hu, 2014; Kadhum and Shamma, 2017). Thus, elaidic acid (EL), the most abundant *trans*-monoene contained in hydrogenated vegetable oil, results from the isomerization of OL; palmitelaidic acid, another *trans*-monoene, from the isomerization of its natural precursor palmitoleic acid; and linoelaidic acid, the most abundant *trans*-polyunsaturated FA, from the isomerization of linoleic acid (Tzeng and Hu, 2014, Kadhum and Shamma, 2017). Another source of *trans*-unsaturated FAs are the milk and body fat of ruminants (such as cattle and sheep) that contains up to 8% of *trans* fats (Craig-Schmidt, 2006). *Trans*-vaccenic acid (VA) is the predominant *trans*-monoene formed during rumination, as a result of bacterial fermentation processes (Lock and Bauman, 2004).

Regardless of their origin, *trans*-unsaturated FAs are considered to have negative effects on human health, especially at the cardiovascular level (Brostow et al., 2012). Thus, the nutritional uptake or the plasma concentration of *trans*-unsaturated FAs are positively correlated with the severity of coronary arteriosclerosis (Hadj Ahmed et al., 2018), inflammatory biomarkers (Mazidi et al., 2017), as well as shortening of telomers in circulating leukocytes, which is a proxy of accelerated aging (Mazidi et al., 2018). Accordingly, *trans*-unsaturated FAs precipitate cardiovascular disease in animal models (Monguchi et al., 2017). Moreover, FAs may favor signs of inflammation in tissues (Oteng et al., 2017). Based on major epidemiological studies, (Oomen et al., 2001; Mozaffarian et al., 2006) the US Federal Drug Administration has recommended banning all sources of *trans* FAs from human consumption (Brownell and Pomeranz, 2014).

Free FAs including saturated FAs (and to a lesser degree *cis*-unsaturated FAs) are well known for their potential toxicity, a phenomenon that may contribute to the pathogenesis of lipotoxic diseases (Unger, 2002) as well as to the induction of chronic inflammation contributing to metabolic syndrome (Ralston et al., 2017). Free FAs including PA can induce endoplasmic reticulum (ER) stress or even trigger mitochondrial or lysosomal permeabilization, leading to subsequent caspase activation and apoptosis (Li et al., 2008; Malhi and Kaufman, 2011; Szeto et al., 2016). However, less information is available on the specific toxicity of *trans*-unsaturated FAs.

In spite of the recognized chronic toxicity of *trans* fats, the mechanisms explaining why they provoke arteriosclerosis are largely elusive. Autophagy has recently been recognized as a major mechanism to counteract cardiovascular aging and to diminish arteriosclerosis (Torisu et al., 2016; Ho et al., 2017). Based on this consideration, we decided to systematically investigate the capacity of saturated, *cis*-unsaturated and *trans*-unsaturated FAs to modulate autophagy and other cellular stress responses *in vitro*, in cultured human cells. Our results reveal the unexpected finding that *trans*-unsaturated FAs can inhibit autophagy induction by saturated FAs.

2. Materials and Methods

2.1. Cell Culture and Chemicals

Culture media and supplements for cell culture were purchased from Gibco-Life Technologies (Carlsbad, CA, USA) and plasticware from Greiner Bio-One (Monroe, CA, USA). All cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin G sodium and 100 μg/ml streptomycin sulfate at 37 °C under 5% CO_2. All FAs were purchased from Larodan (Malmö, Sweden); golgicide A, rapamycin, thapsgargin and tunicamycin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Each fatty acid was dissolved at a concentration of 100 mM in an appropriate volume of 100% ethanol pre-warmed at 37 °C. The obtained solution was then used to treat the cells, with final concentrations ranging from 125 to 1000 μM. The SCREEN-WELL® Autophagy library (BML-2837) was purchased from Enzo Life Sciences (Farmingdale, NY, USA).

2.2. Yeast Clonogenic Survival Assay

All yeast experiments were carried out in the BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) wild type strain background. Yeast cells were grown in SC medium containing 0.17% yeast nitrogen base (Difco), 0.5% $(NH_4)_2SO_4$ and 30 mg/l of all amino acids (except 80 mg/l histidine and 200 mg/l leucine), 30 mg/l adenine, and 320 mg/l uracil and 2% glucose. All yeast cultures were inoculated from a stationary overnight culture to an OD_{600} of 0.1 and then grown at 28 °C and 145 rpm shaking for the indicated time. Chronological lifespan was assessed as described before (Buttner et al., 2007). In brief: cultures were inoculated from fresh overnight cultures to an OD_{600} of 0.1 with the culture volume being 10% of flask volume. Aliquots were taken to detect clonogenic survival at the indicated time points (starting from day 2, where aging starts) with 500 cells being plated on YPD agar. FAs (purchased as sodium salts from Sigma-Aldrich) were added to growth media from 1% stock solutions in ddH_2O with 10% tergitol shortly before inoculation to a final concentration of 1.8 mM.

2.3. Fly Kaplan-Meier survival assay

Unless otherwise specified standard laboratory breeding was carried out at 25 °C, 65–70% humidity and a 12:12 h light/dark cycle as described previously (Sigrist et al., 2003). Standard fly food was prepared according to the "*Sigrist*" recipe as a semi-defined cornmeal-molasses medium with slight modifications (0.9 l H2O, 4.2 g agar-agar, 12.5 ml molasses, 85 g malt extract, 8.3 g soy, 66.7 g cornmeal, 1.33 g p-hydroxy-benzoic acid methyl ester dissolved in ethanol, 5.25 ml propionic acid). All experiments were performed in *D. melanogaster* isow1118 background (Hazelrigg et al., 1984). Parental flies were transferred to new vials every third day and only 1–3 days old progenitor flies from the F1 generation were used for experiments. The flies were anesthetized on a porous pad by CO_2 application for 8 min as a maximum, separated into males and females and 20–40 flies each were subsequently transferred to a fresh vial. For assessment of fly-lifespans the flies were sex-separated and transferred to small vials (20 flies per vial) containing either standard corn food or fly food supplemented with 0.05% FA. At least 120 flies per sex and per genotype were analyzed to

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