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Supplement of TCA cycle intermediates protects against high glucose/palmitate-induced INS-1 beta cell death

Sung-E Choi [a,1], Youn-Jung Lee [a,1], Geum-Sook Hwang [b,1], Joo Hee Chung [b], Soo-Jin Lee [a], Ji-Hyun Lee [a], Seung Jin Han [c], Hae Jin Kim [c], Kwan-Woo Lee [c], Youngsoo Kim [d], Hee-Sook Jun [e], Yup Kang [a,*]

[a] Institute for Medical Sciences, Ajou University School of Medicine, Suwon, Republic of Korea
[b] Korea Basic Science Institute, Seoul, Republic of Korea
[c] Department of Endocrinology and Metabolism, Ajou University School of Medicine, Suwon, Republic of Korea
[d] Cancer Research Institute, College of Medicine, Seoul National University, Seoul, Republic of Korea
[e] Leegilya Cancer and Diabetes Institute, Gachon University of Medicine and Science, Inchon, Republic of Korea

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ABSTRACT

The aim of this study is to investigate the effect of mitochondrial metabolism on high glucose/palmitate (HG/PA)-induced INS-1 beta cell death. Long-term treatment of INS-1 cells with HG/PA impaired energy-producing metabolism accompanying with depletion of TCA cycle intermediates. Whereas an inhibitor of carnitine palmitoyl transferase 1 augmented HG/PA-induced INS-1 cell death, stimulators of fatty acid oxidation protected the cells against the HG/PA-induced death. Furthermore, whereas mitochondrial pyruvate carboxylase inhibitor phenylacetic acid augmented HG/PA-induced INS-1 cell death, supplementation of TCA cycle metabolites including leucine/glutamine, methyl succinate/α-ketoisocaproic acid, dimethyl malate, and valeric acid or treatment with a glutamate dehydrogenase activator, aminobicycloheptane-2-carboxylic acid (BCH), significantly protected the cells against the HG/PA-induced death. In particular, the mitochondrial tricarboxylate carrier inhibitor, benzene tricarboxylate (BTA), also showed a strong protective effect on the HG/PA-induced INS-1 cell death. Knockdown of glutamate dehydrogenase or tricarboxylate carrier augmented or reduced the HG/PA-induced INS-1 cell death, respectively. Both BCH and BTA restored HG/PA-induced reduction of energy metabolism as well as depletion of TCA intermediates. These data suggest that depletion of the TCA cycle intermediate pool and impaired energy-producing metabolism may play a role in HG/PA-induced cytotoxicity to beta cells and thus, HG/PA-induced beta cell glucolipotoxicity can be protected by nutritional or pharmacological maneuver enhancing anaplerosis or reducing cataplerosis.

Introduction

Insulin deficiency caused by a loss of pancreatic beta cells and a subsequently impaired compensation for insulin resistance contributes toward the development of type 2 diabetes [1]. Increased levels of free fatty acids (FFAs)[2] are believed to induce a beta cell loss in type 2 diabetic patients, and this was termed 'lipotoxicity'. Since FFAs in conjunction with hyperglycemia potentiates the lipotoxicity, the high glucose/FFA-induced toxicity was an augmented form of lipotoxicity and termed 'glucolipotoxicity' [2]. In fact, long-term exposure to FFA induced beta cell death in culture and in isolated islets [3], and elevated levels of glucose augmented this FFA-induced cell death [4]. This *in vitro* beta cell death by elevated FFA and high concentration of glucose is considered to represent *in vivo* glucolipotoxicity. Cell death was mainly apoptotic with cytochrome c release, caspase 3 activation, and DNA fragmentation [3]. Saturated fatty acids such as palmitic and stearic acids are generally cytotoxic to beta cells, while unsaturated fatty acids like linoleic, oleic, and palmitoleic acids, are not, and actually protect cells from saturated FFA-induced death [5].

The molecular and cellular mechanisms involved in FFA-induced beta cell death as well as the potentiation of FFA-induced death by glucose are not fully understood. Ceramide and the ceramide-induced up-regulation of nitric oxide (NO) have been suggested to be important mediators of FFA-induced beta cell death

* Corresponding author. Address: Institute for Medical Science, Ajou University School of Medicine, Wonchon-dong san 5, Yongtong-gu, Suwon, Gyeonggi-do 442-749, Republic of Korea. Fax: +82 31 219 4482.
E-mail address: kangy@ajou.ac.kr (Y. Kang).

[1] These authors contributed equally to this work.

[2] *Abbreviations used:* CPT-1, carnitine palmitoyl transferase-1; ER, endoplasmic reticulum; FFA, free fatty acid; FAO, fatty acid oxidation; GDH, glutamate dehydrogenase; LC-CoA, long-chain acyl-coenzyme A; PPAR-α, peroxisome proliferator-activated receptors-alpha; ROS, reactive oxygen species; PARP, poly-(ADP-ribose) polymerase; PC, pyruvate carboxylase; TC, tricarboxylate carrier; TCA, tricarboxylate acid; TG, triacylglycerol.

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[3,5–7]. The involvement of NO-mediated mitochondrial DNA damage in FFA-induced INS-1 beta cell death was reported [8]. On the other hand, FFA-induced activation of novel protein kinase Cs (PKCs), which included PKC-δ, was suggested to contribute to beta cell death [9,10]. Activation of NF-κB signal was implicated in FFA-induced beta cell death since IκB kinase inhibitor was found to reduce palmitate-induced INS-1 cell death [11]. It was also reported that elevated concentrations of FFAs or high concentration of glucose produced reactive oxygen species (ROS) and the signals generated from oxidative stress might contribute to induction of beta cell glucolipotoxicity [11–13]. Ca^{2+}-dependent mechanisms were suggested to be involved in FFA-induced beta cell death [14,15]. Down-regulation of the IRS/PI3 kinase/Akt signalling pathway was a critical modulator of FFA-induced beta cell death [16]. Recently, induction of endoplasmic reticular (ER) stress was reported to play a critical role in FFA-induced beta cell death [17,18].

Although several cytotoxic mediators for FFA-induced cytotoxicity have been reported, it is not determined how glucose/FFA-mediated metabolic changes induce activation of such cytotoxic signals. Elevated glucose levels were thought to potentiate FFA-induced beta cell toxicity through lipid partitioning [19]. FFAs are transported into the mitochondria via carnitine palmitoyl transferase-1 (CPT-1) as acyl-CoA form and undergo β-oxidation when FFAs are used as the energy source. However, FFA metabolism can be shifted from the oxidation to esterification pathway when the concentration of glucose is simultaneously elevated [19]. The increased flux of glucose and enhanced glycolysis in beta cells promotes anaplerosis, which is the replenishment of tricarboxylate acid (TCA) cycle intermediates, through the activation of pyruvate carboxylase (PC). The enhanced anaplerosis results in elevation of malate and citrate levels in the mitochondria and ultimately promotes cataplerosis, which accelerates the transport of TCA cycle intermediates to the cytosol [20]. The increased cytosolic citrate can be converted to malonyl-CoA through sequential enzymatic reactions involving ATP citrate lyase and acetyl-CoA carboxylase (ACC). Accumulation of malonyl-CoA inhibits CPT-1 activity, thereby switching fatty acid metabolism from oxidation (FAO) to lipid synthesis (Supplemental Fig. 1) [21]. Ultimately, the accumulation of long-chain acyl-CoA (LC-CoA) or lipid signalling molecules such as phosphatidic acid, lysophosphatidic acid, diacylglycerol (DAG), and ceramide were suggested to play a critical role in FFA-induced lipotoxicity and glucose-induced augmentation [19]. In addition to enhancing the malonyl-CoA pathway, high levels of glucose can further promote the lipogenic pathway through induction of the master lipogenic transcription factor, sterol regulatory element-binding protein-1c (SREBP-1c) [22]. Recently, El-Assaad et al. added that early promotion of lipid esterification was major contributors to high glucose/FFA-induced glucolipotoxicity [23].

Although lipid accumulation and subsequent activation of lipid-mediated signals was associated with FFA-induced toxicity to beta cells [24], triacylglycerol (TG) itself is not likely toxic because TG is a biologically inert molecule [25]. In contrast, TG accumulation could provide survival signals to FFA-treated beta cells by sequestrating toxic lipid intermediates [26,27]. Enhanced synthesis of TG through over-expression of stearoyl-CoA desaturase 1 (SCD1) was reported to reduce FFA-induced cytotoxicity [28]. Recently, the TG/FFA cycling, which enhances lipid esterification in conjunction with increased lipolysis, was suggested to act as the mechanism preventing overnutrition-induced glucolipotoxicity [29]. Enhanced TG/FFA cycling was postulated to divert toxic glucose/FFA metabolism to a useless energy exhaustion metabolism [30]. Collectively, it is not certain whether the promotion of lipid synthetic pathways by exposure to high glucose and FFA plays a critical role in glucolipotoxicity. Recently, a report that incomplete fatty acid oxidation through depletion of TCA cycle intermediates contributed to FFA-induced insulin resistance suggest that mitochondrial dysfunction, rather than activation of lipid-mediated signals, may be a direct mediator for FFA-induced toxicity to beta cells [31]. Furthermore, decrease of various mitochondrial enzymes such as pyruvate carboxylase, glutamate dehydrogenase, isocitrate dehydrogenase, and ATP synthase in beta cells under hyperglycemic and hyperlipidemic conditions supports that mitochondrial dysfunction through metabolic failure may be a critical contributor to high glucose/FFA-induced cytotoxicity to beta cells [23,32,33].

The present study was initiated to determine whether metabolic impairment in mitochondria was involved in high glucose/FFA-induced glucolipotoxicity to beta cells and whether the maintenance of TCA cycle intermediate pool could protect against the toxicity. We initially tested whether mitochondrial energy-producing metabolism was impaired in high glucose/palmitate (HG/PA)-treated INS-1 beta cells. We measured the level of intracellular ATP and the oxidation rate of glucose and palmitate and determined the levels of TCA cycle intermediates in the HG/PA-treated cells. We next investigated the role of glucose/FFA metabolism in HG/PA-induced cytotoxicity using modulators of anaplerosis and cataplerosis as well as modulators of fatty acid oxidation. The effect of reduced anaplerosis and cataplerosis on HG/PA-induced INS-1 cell death was examined through gene knockdown of glutamate dehydrogenase (GDH) and mitochondrial tricarboxylate carrier (TC), respectively. We finally investigated whether GDH activator and TC inhibitor would be able to restore the HG/PA-induced reduction of oxidation metabolism and depletion of TCA cycle intermediates.

Materials and methods

Reagents

All chemicals, including glucose, palmitate, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoilium bromide (MTT), bezafibrate, etomoxir, phenylacetic acid (PAA), T0901317, fumonisin B1, myriosin, GF109203X, chelerythrine, N-acetylcysteine (NAC), reduced glutathione (GSH), methyl pyruvate, leucine, glutamine, monomethyl succinate, α-ketoisocaproate, dimethyl malate, valeric acid, 2-aminobicyclo[2.2.1]heptan-2-carboxylic acid (BCH), 2,3-benzenetricarboxylate (BTA), and ATP were purchased from either Merck Bioscience (Darmstadt, Germany) or Sigma–Aldrich (St. Louis, MO). The chemicals were dissolved in either appropriate media solution or dimethyl sulfoxide (DMSO) and then treated at the required working dilution. All chemicals were handled in accordance with the supplier's recommendations. Anti-caspase 3 and PARP antibodies were purchased from Cell Signalling Technology (Beverly, MA).

Preparation of palmitate

Preparation of palmitate was slightly modified from Listenberg's protocol [34]. Palmitate/BSA (bovine serum albumin) conjugates were prepared through soaping palmitate with sodium hydroxide and mixing with BSA. Briefly, a 20 mM solution of palmitate in 0.01 M NaOH was incubated at 70 °C for 30 min and the fatty acid soaps were then complexed with 5% fatty acid-free BSA in phosphate-buffered saline (PBS) in 1:3 volume ratio. The complexed fatty acids consisted of 5 mM palmitate and 3.75% BSA. The palmitate/BSA conjugates were diluted in 10% FBS culture medium (approximately 0.4% BSA) and administered to cultured cells. Molar ratio of palmitate to BSA in 0.4 mM palmitate is 3.5:1 and the concentration of BSA is approximately 0.7%.

Cells

INS-1 rat insulinoma cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad,