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Original Contribution

Fatty acid-mediated intracellular iron translocation: A synergistic mechanism of oxidative injury

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Abstract

Fatty acid has been reported to be associated with cardiovascular diseases and cancer, but the possible mechanism remains unclear. Here, we reported a novel mechanism for the permissive role of fatty acid on iron intracellular translocation and subsequent oxidative injury. In vitro study from endothelial cells showed that iron alone had little effect, whereas in combination with PA (palmitic acid), iron-mediated toxicity was markedly potentiated, as reflected in mitochondrial dysfunction, cell death, apoptosis, and DNA mutation. We also showed that PA not only facilitated iron translocation into cells through a transferrin-receptor (TfR)-independent mechanism, but also translocated iron into mitochondria; the subsequent intracellular iron overload resulted in reactive oxygen species (ROS) overgeneration and lipid oxidation. Further investigation revealed that PA-facilitated iron translocation is due to Fe/PA-mediated extracellular oxidative stress and the subsequent membrane damage with increased membrane permeability. Fe/PA-mediated toxic effects were reduced in ρ0 cells lacking mitochondrial DNA or by antioxidant enzyme SOD, especially mitochondrially localized MnSOD, suggesting a permissive role of PA for iron deposition on the vascular wall and its subsequent toxicity via mitochondrial oxidative stress. This observation was confirmed in vivo in mice, wherein higher vascular iron deposition and accompanying superoxide release were observed in the presence of a high-fat diet with iron administration.

Keywords: Free fatty acids; Palmitic acid; Mitochondria; Cancer; Apoptosis; Iron overload; Oxidative stress; Lipid oxidation; Free radical

Introduction

Several epidemiological studies, or animal and individual-based studies, have reported that dietary fat intake is associated with increased risk of cardiovascular diseases [1–3] and cancer [4–6], but the conclusion remains controversial, indicating that an as-yet-unidentified factor may modulate these effects. In this study, we found that fatty acid plays a permissive role for iron deposition on the vascular wall when the iron was absorbed and released into plasma, and the subsequent iron-mediated oxidative injury may explain the involvement of fatty acid on cardiovascular diseases and cancer.

Free fatty acids (FFA) are involved in mitochondrial function [7] and have been associated with several patho-

Abbreviations: AFC, Ac-DEVD-7-amino-4-trifluoromethylcoumarin; anti-FABP, anti-plasma membrane-bound fatty acid binding protein; BSA, bovine serum albumin; DCFDA, 2′,7′–dihydrochlorofluorescein-diacetate; DFX, deferoxamine mesylate; $DiOC_6$, 3,3′-dihexiloxadicarbocyanine; DMSO-TBAC, dimethyl sulfoxide–tetrabutylammonium chloride; FFA, free fatty acids; HUVECs, human umbilical vein endothelial cells; JC-1, 5,5′,6,6′-tetrachloro-1,1′3,3′-tetraethyl-benzimidazol-carbocyanine iodide; MMP, mitochondria membrane potential; mtDNA, mitochondria DNA; nDNA, nuclear DNA; MTT, 3-(4,5-dimethylthianol-2-yl)-2,5 diphenyltetrazolium bromide; PA, palmitic acid; PBS, phosphate-buffered saline; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; TfR, transferrin receptor; TfRab, monoclone anti-transferrin receptor antibody.

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logical conditions, including cardiovascular disease and cancer [8]. FFA affect cell membrane function [9,10] and have been shown to cause mitochondrial injury, as reflected in mitochondrial swelling [11], uncoupling of oxidative phosphorylation [12], permeability transition pore opening [13], and cell death [14]. The deleterious effects of FFA appear to depend on chain length and degree of saturation [8]; however, the exact mechanisms as to how they affect cell injury are poorly understood. It has been reported that under certain conditions, FFA can form complexes with and shuttle iron across the lipid bilayer membrane [15]. Other studies demonstrate that saturated FFA, such as palmitic acid (PA) can translocate iron into intact human erythrocytes [16], suggesting that fatty acid could play a role in the process of cellular iron deposition and toxicity.

Iron is an important nutritional component, which is widely contained in blood, muscle tissue, and some critical enzymes. In cases of excessive iron intake, iron can be highly toxic to biological molecules as an active oxidizing agent. However, some aspects of iron transport and the precise mechanisms for iron-mediated cellular toxicity are still incompletely understood [17]. It has been indicated that the intracellular iron level is controlled by the cell surface transferrin receptor (TfR)-mediated iron uptake as transferrin iron [18,19]. Under normal conditions, iron in the serum is primarily transported bound to carrier proteins (e.g., transferrin and lactoferrin); iron is sequestered and detoxified within cells by binding to ferritin and haemosiderin, the principal iron-storage proteins [20]. Some iron in the circulation is also loosely bound to citrate, lactate, phosphate, albumin, or other proteins. When the availability of the labile iron pool increases [21], iron-mediated toxicity is enhanced under pathological situations. The toxic effects of iron overload have been shown to involve oxidative reactions and redox cycling of the metal [22]. However, the reducing and oxidizing equivalents involved in this cycling might arise from a number of intracellular sources, among which reactive oxygen species (ROS) generated by active mitochondria may be of particular importance [23]. Mitochondria are intrinsically rich in iron, which is required for the proper function of iron-containing enzymes such as cytochrome *c* oxidase and iron–sulfur proteins (e.g., complexes I, II, and III and aconitase). Thus, changes in the amount of intracellular iron or FFA could impair mitochondrial membrane function and the electron transport chain, and thereby determine the degree of cellular injury [24].

In an effort to define the synergistic effect of fatty acid on iron-mediated oxidative injury, palmitic acid, a major component of FFA in plasma [25] was employed to study the permissive role of fatty acid on vascular iron deposition in human umbilical vein endothelial cells (HUVECs). Our results demonstrate that PA markedly increases iron-mediated toxicity, including apoptosis, DNA mutation, and cell death. We also showed that PA enhances iron translocation into cells and further into mitochondria, which mainly result in mitochondrial oxidative stress. Further investigation found that PA-facilitated iron translocation is due to Fe/PA-mediated extracellular oxidative stress and the subsequent membrane damage with increased membrane permeability. This effect was further proved by dietary fat intake through in vivo experiments in mice.

Experimental procedures

Cells, materials, and methods

HUVECs cell line CRL-1730 (obtained from ATCC) [26] was conditionally immortalized by hTERT expression (from Gene Engineering Center in Tongji Medical College) with an extended life span [27,28], which is sufficient for preparation of ρ0 cells and the retroviral-carried SOD overexpression-stable cell lines. Cells were cultured in Ham's F12K medium with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 0.1 mg/ml heparin, 0.03–0.05 mg/ml endothelial cell growth supplement (ECGS), and 10% fetal bovine serum at 37°C in 5% CO_2. HUVECs ρ0 cells (lacking mitochondrial DNA) were cultured in the same medium supplemented with 200 μg/ml sodium pyruvate and 150 μg/ml uridine. Ferrous ammonium sulfate stock solutions were prepared freshly in degassed, argon-purged Krebs buffer in the presence of 0.1 mM ascorbic acid. Palmitic acid was dissolved in ethanol following the addition of bovine serum albumin (BSA, 2.5%, dissolved by PBS buffer) and the ethanol was subsequently evaporated in N_2 gas by continuous stirring. Identical amounts of ascorbic acid, ammonium sulfate, and BSA were used as control. Cells were grown to 80% confluence, starved with serum-free medium for 12 h, and then treated with Fe/PA at concentrations of 0.15/0.3 mM in the presence of 25 μg/ml transferrin and indicated factors for 12 h; the viable cells were used for further analysis. Antioxidants, which include Lazaroid (LAZ) U-74389G (from BIOMOL), a drug for lipid oxidation inhibitor at final concentration of 1 mM [29], a combination of antioxidant enzymes SOD and catalase (SOD/CAT from Sigma) at a final concentration of 1 mg/L, and a combination of antioxidant vitamins C and E (VitC and E from Sigma) at final concentration of 1 mM were used in some experiments as indicated. Protein concentrations were measured by the Bio-Rad protein assay kit (Bradford method) according to manufacturer's instructions using BSA as a standard.

Cell transfection and SOD overexpression

The cDNAs for human MnSOD and Cu/ZnSOD, which were cloned in pRetro-Off expression vectors were described in previous work [30]. HUVECs were transfected with the above vectors using LipofectAMINE kits (Invitrogen) according to the manufacturer's instructions. Transfected cells were grown in puromycin (1.5 nmol/ml) for