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Asymmetrical cross-talk between the endoplasmic reticulum stress and oxidative stress caused by dextrose

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ABSTRACT

Aims: Oxidative and endoplasmic reticulum (ER) stresses are implicated in premature cardiovascular disease in people with diabetes. The aim of the present study was to characterize the nature of the interplay between the oxidative and ER stresses to facilitate the development of therapeutic agents that can ameliorate these stresses.
Main methods: Human coronary artery endothelial cells were treated with varying concentrations of dextrose in the presence or absence of three antioxidants (alpha tocopherol, ascorbate and ebselen) and two ER stress modifiers (ERSMs) (4-phenylbutyrate and taurodeoxycholic acid). ER stress was measured using the placental alkaline phosphatase assay and superoxide (SO) generation was measured using the superoxide-reactive probe 2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-A]pyrazin-3-one hydrochloride chemiluminescence.
Key findings: The SO generation was increased with increasing concentrations of dextrose. The ER stress was increased with both low (0 and 2.75 mM) and high (13.75 and 27.5 mM) concentrations of dextrose. The antioxidants inhibited the dextrose induced SO production while in high concentrations they aggravated ER stress. The ERSM reduced ER stress and potentiated the efficacy of the three antioxidants. Tunicamycin-induced ER stress was not associated with increased SO generation. Time course experiments with a high concentration of dextrose or by overexpressing glucose transporter one in endothelial cells revealed that dextrose induced SO generation undergoes adaptive down regulation within 2 h while the ER stress is sustained throughout 72 h of observation.
Significance: The nature of the cross talk between oxidative stress and ER stress induced by dextrose may explain the failure of antioxidant therapy in reducing diabetes complications.

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1. Introduction

Oxidative stress and endoplasmic reticulum (ER) stress have been suspected to be major contributors to the accelerated coronary artery disease (CAD) in diabetes [1–4]. In endothelial cells, dextrose induces the generation of reactive oxygen species leading to the expression of vascular adhesion molecules and chemoattractants [1–4]. This oxidative load is counterbalanced by a host of enzymatic and non-enzymatic antioxidants [5].

Despite the robust experimental evidence for a potential role of oxidative stress in CAD, the available interventional trials have mostly failed to show any salutary effects of antioxidant vitamins in preventing cardiovascular events [5,6]. The precise cause of this apparent paradox is not known. It has been suggested that the lack of salutary effects of antioxidant vitamins on ER stress and their potential for promoting reductive stress may have limited their clinical effectiveness [6,7]. Since therapeutic agents that target oxidative stress could alter the milieu of various cellular compartments, it is imperative to understand the interplay between various cellular stresses.

In this communication a series of experiments with antioxidants and ER stress modifiers (ERSMs) show that antioxidants inhibit the dextrose induced SO production in endothelial cells while at high concentrations they aggravate ER stress. In contrast, the ERSMs potentiate the efficacy of antioxidants despite of their lack of intrinsic antioxidant activity.

2. Materials and methods

2.1. Materials

The chemiluminescent alkaline phosphatase substrate 3-(diethylamino)-1-(2,2-dimethyl-3-H-1-benzofuran-7-yl)-propan-1-one (CSPD) was purchased from Clontech Laboratories, Inc. (Mountain View, CA). Lipofectamine and the superoxide-reactive probe 2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-A]pyrazin-3-one hydrochloride (MCLA) were obtained from Life Technologies (Carlsbad, CA) All other chemicals were obtained from Sigma-Aldrich Corporation (Saint Louis, MO) or Fisher Scientific (Pittsburgh, PA).

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2.2. Cell culture

Human coronary artery endothelial cells (HCAECs) were purchased from American Type Culture Collection (ATCC) (Manassas, VA) and cultured in Vascular Cell Basal Medium (ATCC) supplemented with 0.2% bovine brain extract, 5 ng/ml recombinant human epidermal growth factor, 10 mM glutamine, 1 μg/ml hydrocortisone hemisuccinate, 0.75 units/ml heparin sulfate, 50 μg/ml ascorbic acid, 2% fetal bovine serum, 10 units/ml penicillin and 10 μg/ml streptomycin. The cells were housed in a humidified incubator at 37 °C and 5% CO_2. Cell viability in all experiments were monitored by measuring lactate dehydrogenase (LDH) in the conditioned medium as described previously [8]. Using this parameter of cell viability, there was no evidence of cell death at any time following treatment with various compounds including during experiments when dextrose concentrations in the culture media were at an extreme (i.e. 0 mM and 27.5 mM). To account for possible effects of osmolarity, select experiments were carried out in the presence of 27.5 mM 2-deoxyglucose and the cells were found to behave like the control cells grown in 5.5 mM dextrose.

2.3. ER stress measurement

ER stress was measured using the placental alkaline phosphatase (ES-TRAP) assay [9–14] (Great EscAPe™, Clontech Laboratories, Inc.). Briefly, HCAECs at 70–80% confluence in six-well plates were transfected with the plasmid pSEAP2-control, containing a truncated placental alkaline phosphatase gene adjacent to the simian virus 40 early gene promoter, and 24 h later treated with varying concentrations (0 to 27.5 mM) of dextrose with various test compounds or the solvent. Under similar transfection conditions, transfection of a plasmid expressing the green fluorescent protein demonstrated that 15–20% of the cells were transfected (data not shown). After 24 h, secreted alkaline phosphatase (SAP) activity was measured as described previously [9–14]. The solvent DMSO (diluted 1:1000) had no effect on SAP activity. The decrease in SAP activity as a measure of ER stress is a convenient and robust method suited for the large number of measurements required in dose–response and time–course studies [9]. The ES TRAP assay has been validated in our laboratory against other known assays for measuring the unfolded protein response (UPR) such as glucose regulated protein 78 induction, phosphorylation of c-*jun*-N-terminal kinase (JNK), and phosphorylation of eukaryotic initiating factor 2α (eIF2α) (measured by Western blotting), and measurement of X-box binding protein 1 (XBP1) mRNA splicing using reverse transcriptase-polymerase chain reaction [13,14].

2.4. Superoxide generation

Superoxide (SO) anion generation was measured using MCLA chemiluminescence as previously described [11–17]. Confluent

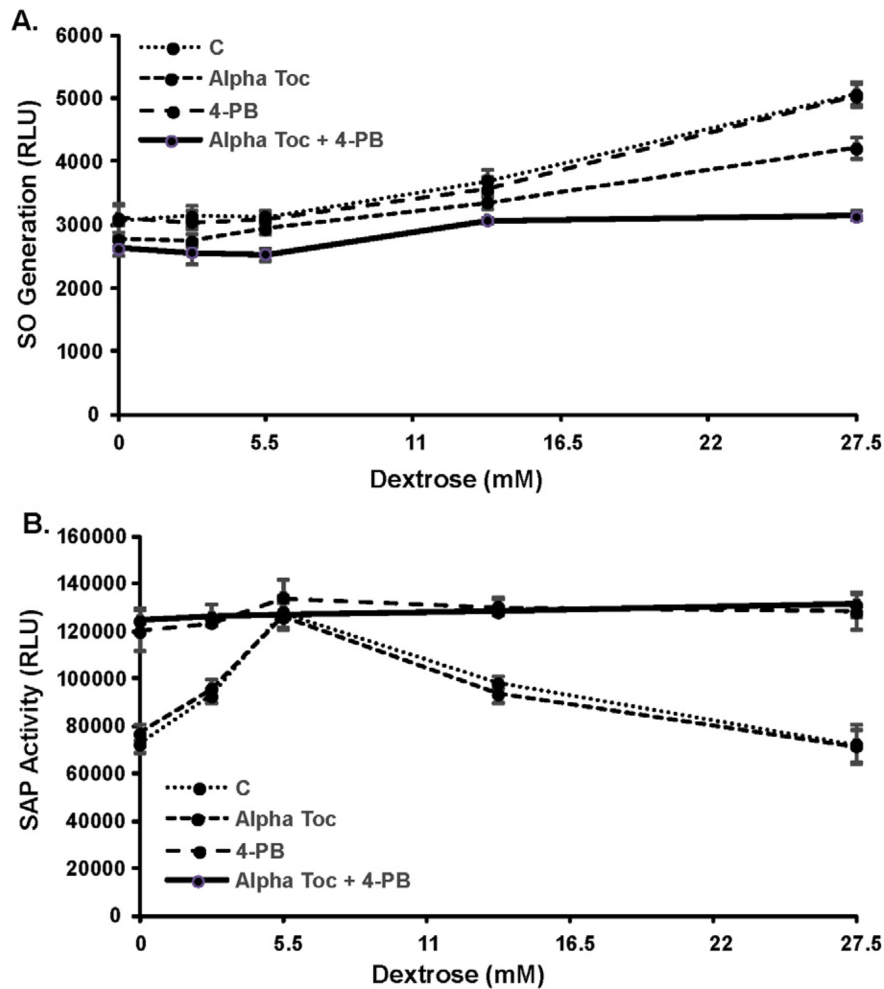

Fig. 1. The effect of α-tocopherol (α-Toc) and 4-phenylbutyrate (4-PB) on superoxide (SO) anion generation and endoplasmic reticulum (ER) stress in HCAECs. Cells were cultured in media containing 0, 2.75, 5.5, 13.75, and 27.5 mM dextrose with and without 1 mM α-Toc and 10 μM 4-PB. A. Dextrose induced a dose-dependent increase in SO anion generation in the control cells. Addition of 4-PB alone had no effect on SO anion generation while addition of α-Toc decreased SO anion generation. Addition of α-TOC and 4-PB combined decreased SO anion generation greater than α-TOC alone. B. Cells were transfected with the plasmid pSEAP2-control and 24 h later placed in media containing 0, 2.75, 5.5, 13.75, and 27.5 mM dextrose. After 24 h, SAP activity was measured. The ER stress is measured as the reduction in secreted alkaline phosphatase (SAP) activity. Both low dextrose and high dextrose concentrations induced ER stress. Addition of 1 mM α-TOC had no effect on ER stress, however addition of 10 μM 4-PB with and without α-TOC prevented ER stress.

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