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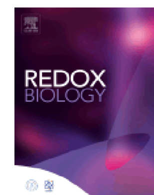

Research paper

The Bioenergetic Health Index is a sensitive measure of oxidative stress in human monocytes

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ABSTRACT

Metabolic and bioenergetic dysfunction are associated with oxidative stress and thought to be a common underlying mechanism of chronic diseases such as atherosclerosis, diabetes, and neurodegeneration. Recent findings support an emerging concept that circulating leukocytes and platelets can act as sensors or biomarkers of mitochondrial function in patients subjected to metabolic diseases. It is proposed that systemic stress-induced alterations in leukocyte bioenergetics are the consequence of several factors including reactive oxygen species. This suggests that oxidative stress mediated changes in leukocyte mitochondrial function could be used as an indicator of bioenergetic health in individuals. To test this concept, we investigated the effect of the redox cycling agent, 2,3 dimethoxynaphthoquinone (DMNQ) on the bioenergetic profiles of monocytes isolated from healthy human subjects using the extracellular flux analyzer. In addition, we tested the hypothesis that the bioenergetic health index (BHI), a single value that represents the bioenergetic health of individuals, is dynamically sensitive to oxidative stress in human monocytes. DMNQ decreased monocyte ATP-linked respiration, maximal respiration, and reserve capacity and caused an increase in proton leak and non-mitochondrial respiration compared to monocytes not treated with DMNQ. The BHI was a more sensitive indicator of the DMNQ-dependent changes in bioenergetics than any individual parameter. These data suggest that monocytes are susceptible to oxidative stress mediated by DMNQ and this can be accurately assessed by the BHI. Taken together, our findings suggest that the BHI has the potential to act as a functional biomarker of the impact of systemic oxidative stress in patients with metabolic disorders.

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1. Introduction

Mitochondria are both a source and target for oxidative stress and the failure to maintain mitochondrial quality by the appropriate balance of biogenesis and autophagy ultimately leads to cell death [1–4]. Many of the chronic diseases which afflict aging populations, such as diabetes and atherosclerosis, are associated with both increased systemic oxidative stress and mitochondrial dysfunction [1,3,5,6]. Translation of these ideas to the clinic in diseases such as diabetes and cardiac surgery has resulted in the hypothesis that circulating platelets or monocytes can serve as bioenergetic biomarkers of the systemic exposure to metabolic stressors or pro-inflammatory cytokines [7–11]. Indeed, a broad range of environmental, dietary and epidemiological studies have shown distinct patterns of cytokine profiles in patients with chronic inflammatory diseases such as cancer, diabetes, obesity, and metabolic syndrome [12–15]. Another study reported that human mononuclear cells isolated from Type 2 diabetic patients have alterations in mitochondrial morphology, mitochondrial mass and membrane potential [16]. These data highlight how measurement of cellular bioenergetics in leukocytes and platelets can act as a surrogate index of mitochondrial function in several pathologies including Alzheimer's disease or in some cases can be directly related to underlying pathologies such as autoimmune diseases [17–19]. Since mitochondria are particularly susceptible to oxidative stress, we hypothesized that the bioenergetics in circulating monocytes would exhibit a dose dependent change in response to oxidants.

We have recently developed methods to assess the changing dynamics of bioenergetic function in leukocytes and platelets from human subjects [20–22]. Importantly, we have determined that

Abbreviations: AA, antimycin A; BHI, bioenergetic health index; DMNQ, 2,3 dimethoxynapthoquinone; DPI, diphenyleneiodonium chloride; FCCP, carbonyl cyanide p-[trifluoromethoxy]-phenyl-hydrazone; XF, extracellular flux; OCR, oxygen consumption rate; ROS, reactive oxygen species

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both glycolysis and oxidative phosphorylation are functionally distinct in platelets, monocytes, neutrophils and lymphocytes which suggests that their response to oxidative stress will also differ [23]. Mitochondrial cellular function can be defined using a stress test in which the addition of oxidative phosphorylation inhibitors can cause alterations in a cell's oxygen consumption rate (OCR) and bioenergetic profile [24,25]. The mitochondrial parameters from the stress test include ATP linked respiration, proton leak, maximal respiration, the reserve capacity and non-mitochondrial respiration [1]. Each of these parameters are all uniquely sensitive to different free radicals or oxidants. The interactive nature of these parameters has led us to propose that an integrated single value known as the Bioenergetic Health Index (BHI) can be used as a measurement of changing bioenergetic health in human subjects for translational medicine [21]. Specifically, this integrated value could be useful for prognostic or diagnostic value. We have previously shown that the BHI is significantly depressed in monocytes isolated from the post-operative pericardial fluid of patients undergoing cardiac surgery [26] and this is associated with the highly oxidative environment of the pericardial fluid following surgery [11].

In the present study, we tested whether the response of monocytes isolated from healthy human subjects show a differential response to oxidative stress produced by 2, 3 dimethoxynapthoquinone (DMNQ). DMNQ is a redox cycling agent that generates both superoxide and hydrogen peroxide intracellularly in a concentration dependent manner [27]. These two oxidants are known to be generated in a broad range of pathological conditions associated with inflammation due to the activation of the NADPH oxidases [28–30]. The concept that cells isolated from patients may exhibit increased susceptibility to the oxidants generated by DMNQ has recently been tested in subjects with autistic disorder [31]. Specifically, it was determined that lymphoblastoid cells from a subgroup of children with autistic disorder had increased mitochondrial dysfunction due to DMNQ mediated oxidative stress compared to another subgroup of children with autistic disorder. In the present study, we examined the impact of varying concentrations of DMNQ in monocytes isolated from healthy subjects. Additionally, we evaluated the utility of the BHI to detect mitochondrial dysfunction induced by DMNQ in monocytes. Interestingly, the BHI was suppressed with increasing DMNQ concentrations and was found to be a more sensitive parameter than individual parameters derived from the mitochondrial stress test. Our findings suggest that the BHI is dynamically sensitive to acute oxidative stress in human monocytes and could be a potential functional biomarker of oxidative stress in patients suffering from metabolic disorders.

2. Materials and methods

2.1. Materials

DMNQ (2,3 dimethoxynapthoquinone) was purchased from Enzo Life Sciences (Farmingdale, NY, USA). RPMI was obtained from Life Technologies (Grand Island, NY, USA). Materials for the extracellular flux assays were from Seahorse Biosciences (North Billerica, MA, USA). BSA, Oligomycin, FCCP (carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone), Antimycin A and diphenyleneiodonium chloride (DPI) were all purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Human subjects

The work described here has been carried out in accordance with the Declaration of Helsinki. All study protocols for the collection and handling of human samples were reviewed and approved by the Institutional Review Board at the University of Alabama at Birmingham. Written informed consent was obtained from all participants. Blood samples (~24 ml) were collected from healthy control subjects (no medications or known pathological conditions, $n=4$–11, 3 males, 8 females, age 30.7 ± 3.6 years) in vacutainers (BD Biosciences, Franklin Lakes, NJ, USA) containing 1.5 ml ACD solution.

2.3. Monocyte isolation from blood

All samples were processed as described previously [20,22]. In brief, the platelet-rich plasma and buffy coat were separated by centrifugation at 500 g for 10 min. The buffy coat was diluted with RPMI media before being applied to a histopaque density gradient to collect peripheral blood mononuclear cells. Afterwards, antibody bead selection and a series of centrifugations were implemented to collect $CD14^+$ monocytes before determining cell counts using the Bio-Rad TC20 automated cell counter (Bio-Rad Laboratories, Hercules, CA, USA). The typical yield of cells from ~24 ml of blood was $5.7 \pm 1.0 \times 10^6$ cells.

2.4. Cellular bioenergetics analysis and the Bioenergetic Health Index (BHI)

The Seahorse Biosciences XF96 extracellular flux analyzer was used to determine the effects of the oxidative stressor, DMNQ, on cellular bioenergetics in human monocytes. Purified monocytes (150,000 cells/well) were resuspended in XF assay buffer (DMEM with 1 mM pyruvate, 5.5 mM D-glucose, 4 mM L-glutamine, pH 7.4) and plated in Cell-Tak (BD Biosciences, Franklin Lakes, NJ, USA) coated assay plates as we have recently described [22]. Cells were pretreated with DMNQ (0.05, 0.1, 0.2, 1 and 5 μM) for 1 h at 37 °C before measuring the oxygen consumption rate (OCR). Dimethyl sulfoxide (DMSO) was used as a vehicle control. The mitochondrial stress test was performed by injecting oligomycin (0.5 μg/mL), FCCP (0.6 μM), and antimycin A (10 μM) sequentially into the cellular media. From this assay we calculated the following parameters: ATP-linked respiration, proton leak respiration, maximal OCR, reserve capacity, and non-mitochondrial respiration [1,25]. The Bioenergetic Health Index (BHI) was calculated using the following formula: BHI = (ATP-linked × reserve capacity)/(proton leak × non-mitochondrial) [21]. In additional experiments, monocytes were treated with DPI (1, 5, and 10 μM) for 15 min prior to being treated with 0.2 μM DMNQ for 1 h and performing the mitochondrial stress test.

2.5. Statistical analysis

The statistical analysis was performed using GraphPad Prism software (La Jolla, CA, USA). Data is presented as mean ± standard error of the mean (SEM). Individual measurements were comprised of 5–6 technical replicates and analyzed as a group by taking the mean of the bioenergetic parameters for each individual. A p value less than 0.05 was considered statistically significant. The statistical significance was determined using a two-tailed paired Student's t-test or ANOVA with Tukey post-hoc test for data with more than two groups as appropriate.

3. Results

3.1. DMNQ alters cellular bioenergetics in monocytes from healthy subjects

To investigate the sensitivity of monocyte mitochondrial function to acute oxidative stress, we utilized the redox cycling agent,

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