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Resveratrol attenuates oxidative stress in mitochondrial Complex I deficiency: Involvement of SIRT3

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ABSTRACT

The pathophysiological mechanisms underlying Complex I (CI) deficiencies are understood only partially which severely limits the treatment of this common, devastating, mitochondrial disorder. Recently, we have shown that resveratrol (RSV), a natural polyphenol, has beneficial effects on CI deficiency of nuclear origin. Here, we demonstrate that RSV is able to correct the biochemical defect in oxygen consumption in five of thirteen CI-deficient patient cell lines. Other beneficial effects of RSV include a decrease of total intracellular ROS and the up-regulation of the expression of mitochondrial superoxide dismutase (SOD2) protein, a key antioxidant defense enzyme. The molecular mechanisms leading to the up-regulation of SOD2 protein expression by RSV require the estrogen receptor (ER) and the estrogen-related receptor alpha (ERRα). Although RSV increases the level of SOD2 protein in patients' fibroblasts, the enzyme activity is not increased, in contrast to normal fibroblasts. This led us to hypothesize that SOD2 enzyme activity is regulated post-translationally. This regulation involves SIRT3, a mitochondrial NAD^+-dependent deacetylase and is critically dependent on NAD^+ levels. Taken together, our data show that the metabolic effects of RSV combined with its antioxidant capacities makes RSV particularly interesting as a candidate molecule for the therapy of CI deficiencies.

1. Introduction

Isolated mitochondrial complex I (CI) deficiency is one of the most common causes of mitochondrial disease. It originates from mutations in either mitochondrial or nuclear DNA [1]. The defect of CI usually causes progressive neuro-degenerative disorders, which explains the variety of clinical presentations associating encephalo-myopathy, developmental delay, hypotonia, seizure, cardiomyopathy, optic atrophy and other organ involvement [2]. Interestingly, CI dysfunction also has been implicated in late-onset neurodegenerative disorders such as Parkinson's disease [3]. In recent years, significant advances have been made in the diagnosis and characterization of these mitochondrial disorders. In particular, the implementation of new sequencing methods has facilitated the identification of numerous disease genes [4]. In contrast, the physiopathology that explains the cellular dysfunctions remains poorly understood which limits treatment options.

Among the possible pathophysiological mechanisms that could link decreases in activity of CI to cellular dysfunction, an imbalance between the production of endogenous reactive oxygen species (ROS) and the antioxidant defense system has been proposed [5]. Although low concentrations of ROS can serve as a second messenger to regulate diverse physiological functions, overproduction of ROS by mitochondria, the main source of intracellular ROS, may lead to oxidative damage. Thus, development of drugs, which can maintain ROS homeostasis, might be useful for alleviating the symptoms associated with CI deficiency [6,7].

Resveratrol is a polyphenolic compound that is proposed to have a wide range of beneficial effects on health [8]. Its numerous biological properties have been studied extensively to understand these effects. In animal models and in cell cultures, resveratrol exerts diverse biological effects probably due to the fact that it has many molecular targets [9]. Recently, we have shown that resveratrol corrects the defects in oxygen consumption in fibroblasts from some patients with moderate CI-deficiency, via the estrogen receptor (ER) and the estrogen-related receptor alpha (ERRα) pathways [10]. Resveratrol also has been shown to exert beneficial

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neuroprotective effects through its antioxidant capacity [11]. However, the precise molecular mechanisms, which underlie the antioxidant effects of RSV, are not completely understood. RSV may have direct scavenging properties or it may induce endogenous anti-oxidative enzymes [11]. Among these anti-oxidative enzymes, the mitochondrial manganese superoxide dismutase (SOD2) is the first-line antioxidant defense enzyme that protects cells from oxidative stress generated in the mitochondria. Sirtuin 3 (SIRT3), a member of the class III histone deacetylase family that resides primarily in mitochondria, may regulate many aspects of oxidative metabolism [12]. Interestingly, several recent studies showed that SIRT3 is a major regulator of the mitochondrial adaptive response to stress, in particular, through activation of SOD2 by deacetylation [13–15]. In this study, we tested the hypothesis that RSV can correct CI deficiency in skin fibroblasts from patients by 1) relieving the metabolic blockade and 2) alleviating oxidative stress. Our results shed new light on the molecular mechanisms underlying these effects.

2. Materials and methods

2.1. Cell culture

Complex I-deficient human fibroblasts were obtained from different reference centers for the investigation and biochemical/molecular diagnosis of mitochondrial disorders. All fibroblasts were obtained from patients with recessively inherited isolated CI deficiency, which originated from mutations in nuclear encoded CI subunit genes, as summarized in Table 1. Unless otherwise mentioned, fibroblasts from patients and normal, control individuals were cultured at 37 °C, 5% CO_2 in RPMI with Glutamax™ (Gibco) supplemented with 10% (V/V) fetal bovine serum and 0.2% (V/V) primocin (Invivogen). For treatment, the medium was removed and vehicle (0.04% DMSO), RSV (75 μM *trans*-RSV, Cayman chemical), TM-RSV (75 μM trimethoxy-resveratrol, Cayman chemical) or RSV with ICI182780 (10 μM Fulvestrant, Sigma) or with XCT790 (5 μM, Sigma) was added to fresh medium for 48 h.

2.2. Oxygen consumption

Maximal Oxygen Uptake Rates (OUR) were measured using Oxoplates®OP96U, 96-well microplates with integrated optical oxygen sensors (PreSens, Germany), as previously described [10].

2.3. Measurement of cellular ATP

Fibroblasts were seeded (4000 cells/well), in triplicate, into 96-well culture plates. The next day, the medium was removed and replaced by glucose-free medium, 10% (V/V) fetal bovine serum, 0.2% (V/V) primocin and supplemented with 5 mM galactose. This procedure reveals putative differences in ATP content, since cells grown in galactose rely, mostly, on oxidative phosphorylation to produce their ATP [16,17]. ATP levels were measured using the Luminescent ATP detection assay kit according to the manufacturer's instructions (Abcam).

2.4. Complex I enzyme activity

Complex I enzyme activity was assayed according to the spectrophotometric method described in [18].

2.5. Measurement of cellular reactive oxygen species

Total levels of cellular ROS were measured using 2′,7′-dichlorodihydrofluorescein-diacetate (H2DCFDA, Invitrogen) according to the manufacturer's recommendations. Briefly, experiments were performed in 24-wells in which cells were incubated with 5 μM H2DCFDA for 40 min at 37 °C. Cells were subsequently washed with PBS, lysed and transferred to a 96-well black plate for measurement of fluorescence intensity with a plate reader (infinite®M200, Tecan). The results were normalized to the amount of protein in each well.

2.6. SIRT3 enzyme activity

Mitochondria were isolated using a mitochondria isolation kit

Table 1
Genotypes of the respiratory CI-deficient patients.

Patients	Mutated gene	Nucleotide changes	Amino acid substitutions	References
P1	*NDUFV1*	c.1142A > G, c.11G > A	p.Gln381Arg, p.Arg386His	This study
P2	*NDUFV1*	c.1156C > T, c.1156C > T	p.Arg386Cys, p.Arg386Cys	[38]
P3	*NDUFV2*	c.54 > A, c.207dup	p.Ala183Thr, p.Tyr70fs*6	This study
P4	*NDUFV2*	c.120+5_120+8delGTAA, c.120+5_120+8delGTAA	p.Gly19_Val40del, p.Gly19_Val40del	This study
P5	*NDUFS1*	c.1139A > T, c.63+6T > G	p.Asp380Val,	This study
P6	*NDUFS1*	c.683T > C, c.755A > G	p.Val228Ala, p.Asp252Gly	[39]
P7	*NDUFS2*	c.1237T > C, c.1237T > C	p.Ser413Pro, p.Ser413Pro	This study
P8	*NDUFS2*	c.875T > C, c.1328T > A	p.Met292Thr, p.Met443Lys	[40]
P9	*NDUFS2*	c.875T > C, c.353G > A	p.Met292Thr, p.Arg118Gln	[40]
P10	*NDUFS4*	c.291delG, c.291delG	p.Trp97X, p.Trp97X	[41]
P11	*NDUFS6*	c. 67del, c. 67del	p.Leu23Trpfs*35, p.Leu23Trpfs*35	This study
P12	*NDUFS7*	c.17-1167C > G, c.17-1167C > G	Ala6_Arg213del, Ala6_Arg213del	[42]
P13	*NDUFS7*	c. 434G > A, c. 434G > A	p.Arg145His, p.Arg145His	[43]