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Antibiotics induce mitonuclear protein imbalance but fail to inhibit respiration and nutrient activation in pancreatic β-cells

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ABSTRACT

Chloramphenicol and several other antibiotics targeting bacterial ribosomes inhibit mitochondrial protein translation. Inhibition of mitochondrial protein synthesis leads to mitonuclear protein imbalance and reduced respiratory rates as confirmed here in HeLa and PC12 cells. Unexpectedly, respiration in INS-1E insulinoma cells and primary human islets was unaltered in the presence of chloramphenicol. Resting respiratory rates and glucose stimulated acceleration of respiration were also not lowered when a range of antibiotics including, thiamphenicol, streptomycin, gentamycin and doxycycline known to interfere with bacterial protein synthesis were tested. However, chloramphenicol efficiently reduced mitochondrial protein synthesis in INS-1E cells, lowering expression of the mtDNA encoded COX1 subunit of the respiratory chain but not the nuclear encoded ATP-synthase subunit ATP5A. Despite a marked reduction of the essential respiratory chain subunit COX1, normal respiratory rates were maintained in INS-1E cells. ATP-synthase dependent respiration was even elevated in chloramphenicol treated INS-1E cells. Consistent with these findings, glucose-dependent calcium signaling reflecting metabolism-secretion coupling in beta-cells, was augmented. We conclude that antibiotics targeting mitochondria are able to cause mitonuclear protein imbalance in insulin secreting cells. We hypothesize that in contrast to other cell types, compensatory mechanisms are sufficiently strong to maintain normal respiratory rates and surprisingly even result in augmented ATP-synthase dependent respiration and calcium signaling following glucose stimulation. The result suggests that in insulin secreting cells only lowering COX1 below a threshold level may result in a measurable impairment of respiration. When focusing on mitochondrial function, care should be taken when including antibiotics targeting translation for long-term cell culture as depending on the sensitivity of the cell type analyzed, respiration, mitonuclear protein imbalance or down-stream signaling may be altered.

1. Introduction

Mitochondria carry their own small genome encoding the mitochondrial 16S and 12S rRNA, 22 tRNAs and 13 proteins of the respiratory chain [24,4]. The large majority of the more than 1200 mitochondrial proteins are therefore transcribed in the nucleus and the proteins imported into the organelle [18]. The respiratory chain complexes are composed of a total of about 90 proteins. The 13 proteins expressed inside mitochondria are essential subunits of complex I, III, IV and V [24,4]. For example complex IV is built from 13 proteins 3 of which COX1, COX2 and COX3 are mtDNA encoded [18,27]. COX1 and COX2 form the catalytic core of complex IV directly involved in the reduction of oxygen the final electron acceptor of the respiratory chain [27].

In order to express the small set of mitochondrial proteins, the organelle has maintained the molecular machineries required for DNA replication, transcription and protein translation [18]. To form the mitochondrial ribosome alone, 78 ribosomal proteins must be imported from the cytosol into the mitochondrial matrix [20]. The mitochondrial ribosome (55S) is structurally quite distinct from eukaryotic cytoplasmic (80S) or bacterial (70S) ribosomes [20,26]. For instance, human mitochondrial ribosomes lack several RNA stem structures of bacterial

Abbreviations: AA, antimycin A; DMSO, dimethyl sulfoxide; FCS, fetal calf serum (heat-inactivated); INS-1E, rat insulinoma cell line clone 1E; KRBH, Krebs-Ringer bicarbonate HEPES buffer; OCR, oxygen consumption rate; Rot, rotenone; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; YC3.6, fluorescent protein yellow cameleon version 3.6

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rRNA but rely on a larger number of ribosomal proteins to form the translation machinery. These differences are surprising as mitochondria are thought to be derived from alpha-proteobacteria [1,26] and their ribosomes are sensitive to several classes of antibiotics targeting the bacterial ribosome at different steps during the peptide elongation cycle [10,12,14,17,2,21,31]. Mitochondrial and bacterial ribosomes may be sensitive to the same molecular entities as the general molecular mechanisms of protein biosynthesis are shared and therefore key nucleotides in the rRNA and core amino acid sequences of ribosomal proteins are conserved [20,3,31,9].

Tetracyclines such as doxycycline for example bind to the 30S bacterial ribosomal subunit and prevent accommodation of amino acid-tRNAs to the acceptor site on the bacterial ribosome thereby blocking peptide elongation [5]. Tetracycline also slow mitochondrial protein synthesis dramatically, lowering the expression of mitochondrial but not nuclear encoded proteins [10,12,15,17]. This inhibition of mitochondrial translation lowers respiratory rates and slows proliferation in mammalian cells [12,19]. Reduced expression of mtDNA encoded proteins with normal unchanged expression of nuclear encoded respiratory chain complex subunits has been termed mitonuclear protein imbalance [10]. The stoichiometric imbalance between mitochondrial and nuclear encoded respiratory chain subunits leads to the mitochondrial unfolded protein response [13]. This processes affects cell function beyond respiration. An impressive example is the extension of lifespan in C. elegans, which is observed when the mitochondrial unfolded protein response is induced during development [10].

Chloramphenicol uses a distinct mechanism blocking the nascent peptide chain by interfering with peptide bond formation on the bacterial ribosome [31]. Chloramphenicol also specifically lowers mitochondrial translation and reduces respiratory rates without affecting the expression of nuclear encoded imported mitochondrial proteins [10,17,21].

Streptomycin and gentamicin are aminoglycosides members of another commonly used class of antibiotics. They bind near the acceptor site on ribosomes, where they cause codon misreading and inhibition of tRNA mRNA complex translocation [16,9]. The gentamicin binding site on the rRNA is homologous between bacteria and mitochondria. This region on the mitochondrial rRNA is susceptible to gentamicin albeit at higher concentrations of the antibiotic [9]. As a consequence gentamycin is able to lower mitochondrial translation and proliferation in mammalian cells [12,2].

Given their effect on mitochondrial translation antibiotics targeting the ribosome can have side-effects in human. One such well-described side-effect is a measurable sign of hearing loss in 20% of patients treated with aminoglycosides [11,9]. A link with mitochondria has been firmly established as specific mitochondrial rRNA mutations in human have been shown to dramatically increase the sensitivity to aminoglycosides [9].

Another cell type strongly dependent on mitochondria is the pancreatic β-cell [30]. This is illustrated for example by maternally inherited diabetes and deafness caused by a heteroplasmic mtDNA mutation in the gene encoding the leucine tRNA [28]. The diabetes phenotypes in these patients is due to a progressive loss of β-cell function and viability.

The main function of the pancreatic β-cell is to secrete the blood glucose lowering hormone insulin [22]. For proper regulation of hormone secretion, the β-cell senses a large number of signals reflecting the nutritional status. Mitochondria are essential in β-cells, linking nutrient sensing and metabolism to insulin granule exocytosis [30]. Mitochondria are required for oxidative metabolism of glucose and amino acids and are generating metabolic signals triggering and amplifying insulin exocytosis. Mitochondrially generated ATP is the main signal of the triggering pathway as it induces closure of plasma membrane K_{ATP} channels. The associated depolarization of the plasmamembrane opens voltage-dependent calcium channels leading to Ca^{2+} rises that trigger insulin granule exocytosis [22,29,6]. Based on this strong reliance of β-cells on mitochondria for energy homeostasis and signal generation for insulin secretion, we speculated that antibiotics by impairing mitochondrial translation of respiratory chain complex subunits should reduce mitochondrial activation of respiration by nutrients also blocking metabolism-secretion coupling. Here we study antibiotics targeting mitochondrial protein synthesis and their ability to affect respiration in INS-1E cells and primary β-cells in the context of the human islet.

2. Materials and methods

2.1. Reagents

Chemicals were from Sigma-Aldrich (Switzerland), Invitrogen (Switzerland), Thermo Fisher Scientific (Waltham, MA, USA), VWR (Switzerland) or Tocris (Switzerland) unless otherwise indicated. The $YC3.6_{cyto}$ pcDNA3 construct (22) was kindly provided by Prof. A. Miyawaki (Riken Brain Science Institute, Wako, Japan).

2.2. INS-1E cell culture

INS-1E cells were obtained from Prof. C. Wollheim and Prof. P. Maechler (University of Geneva). INS-1E cells were cultured at 37 °C in a humidified atmosphere (5% CO_2) in RPMI-1640 medium (GIBCO #21875-034) containing 11 mM glucose, supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS; Brunschwig AG, Switzerland), 10 mM HEPES (pH 7.3), 1 mM sodium pyruvate, 50 μM β-mercaptoethanol. For regular culture of INS-1E cells the antibiotics penicillin (50 μg/ml) and streptomycin (100 μg/ml) were included. To study the effects of antibiotics on the expression of mtDNA encoded proteins or respiration of INS-1E cells the antibiotics were removed 2 days before initiation of the experiments.

2.3. HeLa cell culture

HeLa cells are from the European Collection of Authenticated Cell Cultures and cultured at 37 °C in a humidified atmosphere (5% CO2) in DMEM medium containing 25 mM glucose, L-glutamine without pyruvate (GIBCO #41966-029). The medium was complemented with 10% (v/v) FCS (Chemie Brunschwig, Switzerland). Depending on the conditions used penicillin (50 μg/ml) and streptomycin (100 μg/ml) were added to the medium.

2.4. PC12 cell culture

PC12 rat pheocromocytoma cells were obtained from the European Collection of Authenticated Cell Cultures and cultured at 37 °C in a humidified atmosphere (5% CO_2) in RPMI-1640 medium (GIBCO #21875-034) containing 11 mM glucose and supplemented with 7.5% fetal bovine serum, 7.5% horse serum, penicillin (50 μg/ml) and streptomycin (100 μg/ml).

2.5. Human islet

Human islets were from non-diabetic deceased donors were purchased from Tebu-bio (Le Perray-en-Yvelines, France). Donors had consented to donate organs for medical research and the local independent ethics committee of the Canton of Vaud (Switzerland) had approved the use of these human samples. Islets were cultured at 37 °C in a humidified atmosphere (5% CO_2) in CMRL 1066 medium 5.6 mM glucose without glutamine (GIBCO #21530027). The medium was supplemented with 10% (v/v) heat-inactivated FBS (Chemie Brunschwig AG, Switzerland) and 2 mM L-glutamine. For human islet culture the antibiotics penicillin (50 μg/ml) and streptomycin 100 μg/ml were included in the medium. Upon receipt the islets were allowed to recover for 24 h in complete CMRL 1066 medium before plating them on 804G matrix coated plastic wells (Seahorse XF96 V3 PS Cell