Mitochondrion 9 (2009) 429–437

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Antibiotic effects on mitochondrial translation and in patients with mitochondrial translational defects

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ARTICLE INFO

Article history:
Received 23 March 2009
Received in revised form 8 July 2009
Accepted 4 August 2009
Available online 9 August 2009

Keywords:
Mitochondrial disease
Respiratory chain
OXPHOS
Ribosome
Translation
Antibiotics

ABSTRACT

The infantile presentation of mitochondrial respiratory chain defects frequently simulates acute bacterial infection and sepsis. Consequently, broad spectrum antibiotic therapy is often initiated before definitive diagnosis is reached and without taking into consideration the potential harm of antibiotics affecting mitochondrial translation. Here, we demonstrate that some commonly used translation-targeted antibiotics adversely affect the growth of fibroblasts from patients with defective mitochondrial translation systems. In addition, we show that these antibiotics inhibit mitochondrial translation in vitro. Our results suggest that patients with mitochondrial translation defects may be more vulnerable to toxic-side-effects following the administration of certain translation-targeted antibiotics.

1. Introduction

Many clinically useful antibiotics function by inhibiting the bacterial translational system (Poehlsgaard and Douthwaite, 2005). Typically this is achieved through binding of the antibiotic near the active sites of the ribosome, resulting in alterations that lead to ribosomes that are dysfunctional in translation. Differences, sometimes subtle, between prokaryotic and eukaryotic ribosomes prevent these antibiotics from inhibiting the eukaryotic host translation system to an appreciable extent, making antibiotics a useful therapeutic strategy for combating bacterial infection. In contrast, the translation system of mammalian mitochondria has significant similarities to its bacterial counterpart (Spremulli et al., 2004) and has been shown to be susceptible to inhibition by a number of antibiotics (De Vries et al., 1973; Denslow and O'Brien, 1978; Ibrahim and Beattie, 1973; Ibrahim et al., 1974; McKee et al., 2006; Towers et al., 1972; Zhang et al., 2005). The toxic-side-effects of some antibiotics have been attributed to the inadvertent inhibition of the mitochondrial translation system (Bottger et al., 2001). Despite this finding, the effects of many antibiotics on mitochondrial translation have not been determined and the extent of susceptibility of the mitochondrial translation system to these antibiotics is currently unknown.

Of the 85 polypeptide subunits forming the mammalian mitochondrial respiratory chain complexes, thirteen are encoded by the mitochondrial genome and synthesized by the mitochondrial translation system. The protein components of the mitochondrial translation system, however, are all nuclearly encoded. Recently, in addition to antibiotic susceptibility, it has become clear that mitochondrial translation is sensitive to mutations in nuclearly encoded proteins of the mitochondrial protein biosynthetic system (Bykhovskaya et al., 2004; Edvardson et al., 2007; Miller et al., 2004; Saada et al., 2007; Scheper et al., 2007; Smeitink et al., 2006). In fact, at least 5–10% of infants diagnosed with oxidative phosphorylation (OXPHOS) deficiencies, a common inborn error of metabolism, possess a translational defect (Miller et al., 2004; Sarzi et al., 2007).

The clinical presentation of infants with mitochondrial disorders, such as tachycardia, dyspnea, pallor, hypoactivity, and shock, may also be present in infants with serious bacterial infections such as pneumonia, meningitis, and septic shock. These similarities

Abbreviations: OXPHOS, oxidative phosphorylation; rRNA, ribosomal RNA; tRNA, transfer RNA; EFTu, elongation factor Tu; EFTs, elongation factor Ts; MRP, mitochondrial ribosomal protein; $EFTs_{mt}$, mitochondrial elongation factor Ts; PUS1, mitochondrial pseudouridine synthase 1.

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doi:10.1016/j.mito.2009.08.001

complicate the diagnosis of a mitochondrial disorder in such infants. Moreover, even if a mitochondrial respiratory chain disorder is suspected, a co-existing life threatening bacterial infection cannot be immediately excluded in an ill infant. Furthermore, there may be similarities in biochemical parameters (lactic acidosis, elevated liver enzymes, hypo/hyperglycemia and high levels of urea and creatinine) between bacterial infections and mitochondrial disorders (Enrione and Powell, 2007). Since time is a major factor in the prognosis of infants with bacterial infections, it is a common practice to immediately initiate intravenous administration of broad spectrum antibiotics. Once a bacterial infection is suspected in a newborn, cultures are taken from blood, urine and the cerebrospinal fluid, and treatment with ampicillin and gentamicin is usually initiated to eradicate possible perinatal bacteria including *Escherichia coli*, Group B Streptococci, and *Listeria monocytogenes*. Other types of antibiotics may be used according to the medical conditions and the bacteria. For example, cefotaxime is administered when acute meningitis is suspected, while macrolides are given for possible *Chlamydia* infections. The underlying mitochondrial defect that led to the initial test result is frequently not identified until months following the treatment of a suspected bacterial infection. Therefore, the potential toxic-side-effects of translation-targeted antibiotics on mitochondrial function are not considered.

In this work, we demonstrate that many translation-targeted antibiotics may be particularly problematic for a patient with already compromised mitochondria by examining the effects of several antibiotics on the viability of cells with mitochondrial defects. In addition, we present data showing that many of these antibiotics directly affect the mitochondrial translation system in vitro.

2. Materials and methods

2.1. Subjects

Fibroblasts derived, with informed consent, from four patients with different molecularly-defined defects in mitochondrial translation were included in the study. One patient suffered from a defect in MRPS22, a protein of the mitochondrial ribosomal small subunit. The clinical phenotype was reported elsewhere (Saada et al., 2007); briefly, patients from this family suffered from antenatal edema, presented at birth with severe muscle hypotonia, cardiomyopathy, and tubulopathy accompanied by lactic acidemia. They expired at 2–22 days of age. Of note, one patient was treated with ampicillin and the aminoglycoside gentamicin and another with cefotaxime (Saada et al., 2007).

A second patient suffered from a defect in another mitochondrial ribosomal protein, MRPS16. At 20 weeks of gestation, cerebral ventricular dilatation and agenesis of the corpus callosum were found. At term she was small for gestational age, had non-pitting edema of the limbs and facial dysmorphism (Miller et al., 2004). Amikacin (aminoglycoside) and ampicillin were initiated because of hypothermia, refractory metabolic acidosis, hyperglycemia (15 mmol/L) and leukocytosis (38,000/mm^3). The patient expired at three days of age.

Another patient was previously diagnosed with a mitochondrial translation defect due to a mutation in mitochondrial translation elongation factor Ts ($EFTs_{mt}$, patient 2 in reference Smeitink et al. (2006)). This patient presented on the second day of life with generalized muscle hypotonia and severe metabolic acidosis. Hypertrophic cardiomyopathy, already evident at the first week, caused death at 7 weeks of age. She was intermittently treated with various aminoglycosides, ampicillin, and cefotaxime because of unexplained clinical deteriorations.

The final patient was an Iranian Jewish male suffering from myopathy, sideroblastic anemia, intermittently elevated lactic acid (2–4.6 mM) and mild mental retardation. He was homozygous for a defect in pseudouridine synthase 1 (PUS1), a mitochondrial tRNA modification enzyme (Bykhovskaya et al., 2004). This patient is included in the study as a control since PUS1 is not directly involved in mitochondrial translation, but has a role up-stream.

2.2. Tissue culture and growth measurement

Fibroblasts were grown in a glucose containing permissive medium (Robinson, 1996). Prior to antibiotic treatment, 8×10^3 cells were plated on 96 well or 35 mm tissue culture dishes overnight. The following day the medium was changed to glucose free, restrictive medium in the presence of the various antibiotics (growth in this medium is highly dependent on OXPHOS activity) (Robinson, 1996). It should be noted that initially, a series of experiments varying both the antibiotic exposure time and concentration were performed. These conditions were based on the recommended dosage of each antibiotic in humans. As a result of these findings, subsequent experiments were conducted using the shortest exposure time and the lowest antibiotic concentration that yielded a discernable difference between the control and patient cells. Cells were grown in the presence of 260 μM amikacin (200 μg/ml), 1100 μM streptomycin (800 μg/ml), 230 μM gentamicin (150 μg/ml), 19 μM doxycycline (10 μg/ml), 100 μM tetracycline (77 μg/ml), 260 μM erythromycin (200 μg/ml), 33 μM azithromycin (25 μg/ml), or 310 μM chloramphenicol (100 μg/ml). After a 72 h incubation, growth was assessed by light microscopy and by a colorimetric method based on the staining of basophilic cellular compounds (mainly nucleic acids) with methylene blue basically as described (Pelletier et al., 1988). Briefly, cells were fixed with 0.5% glutaraldehyde for 10 min, rinsed with water, stained with 1% methylene blue in 0.1 M borate buffer, rinsed with water, and allowed to dry. The dye was extracted from the cells with 0.1 N HCl at 37 °C and measured at 622 nm on a microplate ELISA reader (EL809, Bio-Tek instruments Vinooski VT) (Pelletier et al., 1988). The results obtained by methylene blue staining were intermittently verified by performing live counts using trypan blue (data not shown). The % of wild-type or mutant cells grown on galactose compared to glucose was determined in the absence and presence of each antibiotic. The mean % growth and standard deviation from seven replicates were determined. To evaluate the effect of the antibiotics alone, the % growth of the wild-type and mutant cells in the absence of antibiotic were each set to 100% and the % growth in the presence of antibiotic was normalized to these values. Conversely, to determine the cumulative effect of the antibiotic and the mutation together, the % of wild-type cells grown in the absence of antibiotic was set to 100% and all other cell growths were normalized to this value. Error was propagated through the normalization calculations using standard methods (http://phys.columbia.edu/~tutorial/propagation/index.html).

2.3. Preparation of ribosomes, elongation factors, and aminoacyl-tRNA

Bovine $EFTu_{mt}$ was purified basically as described (Bullard et al., 1999), with modifications (Jones et al., 2008). Bovine EFG_{mt} was also purified basically as described (Bhargava et al., 2004), except that following induction at 25 °C overnight, cells were resuspended in a buffer containing 50 mM Tris–HCl, pH 7.6, 40 mM KCl, 7 mM $MgCl_2$, 10% glycerol, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 0.1% Triton X-100 and 7 mM β-mercaptoethanol (βME). The cells were disrupted by sonication on ice for 7 min with alternating 10 s bursts and 50 s cooling periods. The cell lysate was centrifuged at 16,000*g* at 4 °C for 30 min. His(6)-tagged bovine EFG_{mt} was purified from the supernatant using Ni–NTA resin as described (Bhargava et al., 2004) except that the column was washed with 25 ml Buffer C (50 mM Tris–HCl, pH 7.6, 1 M NH_4Cl, 7 mM $MgCl_2$,