The doxycycline paradox in primary mitochondrial diseases

Tamas Kozicz[1,,2,#], Shamima Rahman[3,#] and Eva Morava[1,2,#]

[1]Department of Clinical Genomics, Mayo Clinic, Rochester, MN, USA

[2]University of Pécs Medical School, Pécs, Hungary

[3] Mitochondrial Research Group, UCL Great Ormond Street Institute of Child Health, and Metabolic Unit, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

[#]Equal contribution

Primary mitochondrial diseases (PMDs) are heterogeneous disorders affecting mitochondrial structure, function and/or dynamics and may be caused by pathogenic variants in hundreds of nuclear or mitochondrial DNA encoded genes[1; 2]. PMDs cause pathologies involving virtually any organ and are frequently life-limiting in early life[3; 4]. There are no disease-modifying therapies for the vast majority of PMDs and current treatments are mostly symptomatic[4; 5]. The first multiprotein enzyme complex of the oxidative phosphorylation system is complex I (CI). CI plays an essential role in generation of chemical energy and its deficiency is associated with a robust increase in reactive oxygen species (ROS) production leading to the development of various pathologies including Leigh syndrome[6; 7].

Several mitochondrial functions can be traced back to their endosymbiotic 'bacterial' origin[8]. Consequently, antibiotics that target bacterial translation may also inhibit mitochondrial translation. Historically, some sources suggested avoiding certain antibiotics in patients with PMDs, especially antibiotics affecting translation including doxycycline. A recent Delphi consensus review however found no evidence base for this practice and so it is now discouraged[9].

Interestingly, doxycycline extended lifespan of worms via increasing mitochondrial unfolded protein response and attenuating mitochondrial respiration[10-12]. Specifically, doxycycline dose-dependently reduced oxygen consumption, without affecting citrate synthase activity or ATP levels[10]. Given at a low concentration during development, doxycycline did not appear to cause any adverse effects[10].

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jimd.12531

Through a chemical high-throughput screen in cells derived from patients with PMD, Perry et al. also found that tetracycline analogues including doxycycline rescue cell death and inflammatory signatures through partial and selective inhibition of mitochondrial translation[13]. They also showed that doxycycline treatment strongly promotes fitness and survival of *Ndufs4* knock-out mice, a model of Leigh syndrome[14].

In contrast, Wüst et al. reported that mice treated with doxycycline presented with CI dysfunction and reduced mitochondrial respiratory capacity and concluded that doxycycline impairs mitochondrial function[15]. This may not be surprising since antibiotics designed to target the prokaryotic ribosome are known to induce dysregulation of mitochondrial function[16].

Considering the above observations, doxycycline as a potential drug-targeted therapy for mitochondrial disease is highly paradoxical. So how can one reconcile the seemingly contradictory reports on the beneficiary effects of doxycycline versus its inhibitory action on mitochondrial translation and function?

To solve this paradox, one has to consider the different doses of doxycycline used in the two studies, which are not directly comparable. Another consideration is that doxycycline triggers a metabolic remodeling primarily driven by changes in the fatty acid oxidation pathway, suggesting a switch to lipid breakdown to fuel energy production[10]. Thus, doxycycline induces a metabolic rewiring favoring lipid metabolism[12]. It has also been proposed that in CI deficiencies the activity of CII (succinate dehydrogenase) is significantly upregulated[17]. Increased CII activity as an adaptive response to CI improves lipid oxidation and provides a high rate of ATP[18].

The major electron donor for CI is NADH and $FADH_2$ for CII. The ratio of NADH versus $FADH_2$ oxidation depends on the proton motive force, and lowering proton motive force by *e.g.,* inhibiting complex I by doxycycline will favor $FADH_2$ oxidation, *i.e.,* towards using lipids versus carbohydrate as fuel source.

We propose a mechanism to explain the potential beneficiary effects of doxycycline in individuals with PMDs, especially with CI-related PMD. As doxycycline decreases CI activity,

the accompanying metabolic rewiring favors CII driven fatty acid oxidation and provides a sufficient rate of ATP production to ameliorate symptoms. One has to consider too that this beneficiary effect of doxycycline could be dose dependent.

In summary, doxycycline is another example, along with rapamycin and hypoxia[19; 20], showing that CI activity is not required to rescue pathologies associated with PMDs. Currently, there are no clinically effective therapies for PMDs, and the above findings suggest that tetracyclines might constitute a potential intervention to ameliorate symptoms for PMDs. Further studies are warranted, including clinical trials if additional preclinical data suggest a beneficial effect of doxycycline.

References

1. Wallace, D.C., and Chalkia, D. (2013). Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. Cold Spring Harb Perspect Biol 5, a021220.
2. Rahman, J., and Rahman, S. (2018). Mitochondrial medicine in the omics era. The Lancet 391, 2560-2574.
3. Gorman, G.S., Chinnery, P.F., DiMauro, S., Hirano, M., Koga, Y., McFarland, R., Suomalainen, A., Thorburn, D.R., Zeviani, M., and Turnbull, D.M. (2016). Mitochondrial diseases. Nature reviews Disease primers 2, 1-22.
4. Russell, O.M., Gorman, G.S., Lightowlers, R.N., and Turnbull, D.M. (2020). Mitochondrial Diseases: Hope for the Future. Cell 181, 168-188.
5. Pitceathly, R.D.S., Keshavan, N., Rahman, J., and Rahman, S. (2021). Moving towards clinical trials for mitochondrial diseases. J Inherit Metab Dis 44, 22-41.
6. Koopman, W.J., Willems, P.H., and Smeitink, J.A. (2012). Monogenic mitochondrial disorders. N Engl J Med 366, 1132-1141.
7. Abramov, A.Y., and Angelova, P.R. (2019). Cellular mechanisms of complex I-associated pathology. Biochem Soc Trans 47, 1963-1969.
8. Margulis, L. (1975). Symbiotic theory of the origin of eukaryotic organelles; criteria for proof. Symp Soc Exp Biol, 21-38.
9. De Vries, M.C., Brown, D.A., Allen, M.E., Bindoff, L., Gorman, G.S., Karaa, A., Keshavan, N., Lamperti, C., McFarland, R., Ng, Y.S., et al. (2020). Safety of drug use in patients

with a primary mitochondrial disease: An international Delphi-based consensus. J Inherit Metab Dis 43, 800-818.

10. Houtkooper, R.H., Mouchiroud, L., Ryu, D., Moullan, N., Katsyuba, E., Knott, G., Williams, R.W., and Auwerx, J. (2013). Mitonuclear protein imbalance as a conserved longevity mechanism. Nature 497, 451-457.
11. Zhu, L., Zhou, Q., He, L., and Chen, L. (2021). Mitochondrial unfolded protein response: An emerging pathway in human diseases. Free Radic Biol Med 163, 125-134.
12. Gao, A.W., El Alam, G., Lalou, A., Li, T.Y., Molenaars, M., Zhu, Y., Overmyer, K.A., Shishkova, E., Hof, K., Bou Sleiman, M., et al. (2022). Multi-omics analysis identifies essential regulators of mitochondrial stress response in two wild-type C. elegans strains. iScience 25, 103734.
13. Perry, E.A., Bennett, C.F., Luo, C., Balsa, E., Jedrychowski, M., O'Malley, K.E., Latorre-Muro, P., Ladley, R.P., Reda, K., Wright, P.M., et al. (2021). Tetracyclines promote survival and fitness in mitochondrial disease models. Nat Metab 3, 33-42.
14. Kruse, S.E., Watt, W.C., Marcinek, D.J., Kapur, R.P., Schenkman, K.A., and Palmiter, R.D. (2008). Mice with mitochondrial complex I deficiency develop a fatal encephalomyopathy. Cell metabolism 7, 312-320.
15. Wüst, R.C.I., Coolen, B.F., Held, N.M., Daal, M.R.R., Alizadeh Tazehkandi, V., Baks-Te Bulte, L., Wiersma, M., Kuster, D.W.D., Brundel, B., van Weeghel, M., et al. (2021). The Antibiotic Doxycycline Impairs Cardiac Mitochondrial and Contractile Function. Int J Mol Sci 22.
16. Diaz, F., Enríquez, J.A., and Moraes, C.T. (2012). Cells lacking Rieske iron-sulfur protein have a reactive oxygen species-associated decrease in respiratory complexes I and IV. Molecular and cellular biology 32, 415-429.
17. Lin, Y., Xu, X., Zhao, D., Liu, F., Luo, Y., Du, J., Wang, D., Ji, K., Zhao, Y., and Yan, C. (2020). A novel m.11406 T > A mutation in mitochondrial ND4 gene causes MELAS syndrome. Mitochondrion 54, 57-64.
18. Leverve, X., Batandier, C., and Fontaine, E. (2007). Choosing the right substrate. Novartis Found Symp 280, 108-121; discussion 121-107, 160-104.
19. Ferrari, M., Jain, I.H., Goldberger, O., Rezoagli, E., Thoonen, R., Cheng, K.H., Sosnovik, D.E., Scherrer-Crosbie, M., Mootha, V.K., and Zapol, W.M. (2017). Hypoxia treatment

reverses neurodegenerative disease in a mouse model of Leigh syndrome. Proc Natl Acad Sci U S A 114, E4241-e4250.

20. Johnson, S.C., Yanos, M.E., Kayser, E.-B., Quintana, A., Sangesland, M., Castanza, A., Uhde, L., Hui, J., Wall, V.Z., and Gagnidze, A. (2013). mTOR inhibition alleviates mitochondrial disease in a mouse model of Leigh syndrome. Science 342, 1524-1528.