



## Commentary

## Rapamycin – “One size does not fit all”

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Mitochondrial diseases largely stem from direct or indirect defects in oxidative phosphorylation – the synthesis of ATP using the chemical energy derived from oxidation of nutrients – and result in widely varying and tissue-specific manifestations. Being of genetic and inborn etiology, there are so far no curative therapies. Some of the strategies proposed, and to some extent tested in animal models and in patients, are pharmacological modulation of mitochondrial biogenesis and fission/fusion, and of central cellular pathogenesis-related processes such as apoptosis and autophagy, and metabolic bypass/supplementation therapies [1]. Intense research activities are ongoing to find and test treatment strategies for these devastating disorders. Valuable models for mechanistic studies and preclinical trials have been generated by introducing disease mutations into mice. In this issue, a study testing oral rapamycin in a mouse model (*Coq9<sup>R239X</sup>*) with compromised coenzyme Q (CoQ) biosynthesis, manifesting as encephalomyopathy, showed no beneficial effect [2]. This is contradictory to several previous reports that have shown beneficial effects in mitochondrial disease models [3–8].

Rapamycin is a natural bacterial macrolide metabolite first isolated from a soil sample from Easter Island (Rapa Nui) in 1972. Initially studied as an antifungal agent, its immunosuppressive and antiproliferative properties in mammalian cells were discovered in the 1990s. It (sirolimus) was approved in USA in 1999 for prophylaxis of rejection in renal transplantation patients. Rapamycin binds the 12 kDa FK506-binding protein (FKBP12), and this complex binds and inhibits mammalian target of rapamycin (mTOR, also known as mechanistic TOR) complex (mTORC) [9]. The mTOR nutrient and growth factor signaling pathway is a master regulator of cell growth and metabolism, and therefore implicated in numerous human diseases such as cancer, diabetes, obesity, neurological diseases, and genetic disorders [9]. In 2013, based on their earlier molecular studies in yeast, Johnson et al. first provided evidence that mTOR signaling can be involved in the pathogenesis of mitochondrial diseases [3]. They showed that pharmacological inhibition of the mTOR1 pathway with both oral [4] and intraperitoneally [3] injected rapamycin robustly alleviated encephalopathy and increased survival in *Ndufs4*<sup>-/-</sup> mice, a model of Leigh syndrome with respiratory

chain complex I deficiency. Thus far, six preclinical studies on the effect and efficacy of rapamycin in mouse models of mitochondrial disease have been published ([3–8], Table 1). They show that high doses of rapamycin have beneficial effects in mitochondrial encephalopathy [4,6] and skeletal myopathy [5,8]. In a *Tk2-H126N* knock-in mouse model of mtDNA depletion syndrome with encephalopathy and myopathy, no convincing effect on tissue histopathology was observed upon mTOR1 inhibition with rapamycin, but the survival of the sick mice was extended [7]. The “deletor mouse” (carrying a Twinkle helicase transgene with a dominant patient mutation) presenting with mitochondrial myopathy was reported to have changes in several pathways downstream of mTOR1 (such as folate cycle, serine biosynthesis, FGF21 secretion and fiber-specifically increased S6 phosphorylation) in the affected muscles, thereby indicating rapamycin as a potential treatment. Indeed, i.p. administration of 8 mg/kg resulted in robust improvement of muscle function and histology and normalization of mtDNA deletion load and several other molecular parameters [5]. Likewise, in muscle-specific *Cox15* knockout mice with disrupted respiratory chain complex IV assembly, despite the lack of clear activation of mTOR1 downstream pathways, rapamycin stimulated autophagy and biogenesis of lysosomes, which the authors suggested resulted in improved muscle histology. However, if rapamycin affected the premature lethality of these mice was not reported [8].

The present study by Barriocanal-Casado et al. [2] investigated, in spite of no clear evidence of increased mTOR1 activity in their model, whether rapamycin has a general beneficial effect in mitochondrial encephalomyopathy. They utilized *Coq9<sup>R239X</sup>* mice with defective CoQ biosynthesis and the two oral dosages used, a low and a high (Table 1), corresponded to those previously shown efficacious in other models [4–6]. The outcome measures included survival, motor performance, brain imaging, tissue histology, and transcriptome and metabolite assessments. Apart from slight improvement in cerebral histopathology, rapamycin had no effect on any parameters of disease progression. The low dose resulted in changes of liver metabolites and midbrain transcriptomics, indicating an effect at molecular level. With the higher dose, all examined tissues responded to rapamycin as judged by S6 protein phosphorylation, a widely used and robust indicator of mTOR activity. Unexpectedly, the high dose resulted in an adverse effect on the mice, decreasing their survival. Interestingly, Barriocanal-Casado et al. did not observe induction of autophagy in any tissue analyzed (brain, heart, liver, and kidney) despite clear mTORC1 inhibition by the high

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**Table 1**  
**Preclinical rapamycin trials in mouse models of mitochondrial disease** (\*mTOR1 activation in the mutant verified by analysis of S6 phosphorylation)

| Model [reference]   | Route, dose  | Survival                                       | Histo-pathology            | Motor perform. | mTOR act.*/+rapamycin            |
|---|--|--|----------------------------|----------------|----------------------------------|
| <i>Ndufs4</i> <sup>-/-</sup> encephalopathy [3]                     | i.p., 8.0 mg/kg daily or every other day               | Increased with daily dose                      | Improved                   | Improved       | Increased/decreased              |
| <i>Ndufs4</i> <sup>-/-</sup> encephalopathy [4]                     | oral, 14, 42, 126 or 378 ppm in chow                   | 14 no effect<br>42 increased<br>378 increased  | Not reported               | Not reported   | Not reported                     |
| Dominant mutant <i>Twinkle</i> transgene, myopathy [5]              | i.p., 8.0 mg/kg daily                                  | Not reported                                   | Improved                   | Not reported   | Increased/decreased              |
| <i>Ndufs4</i> <sup>-/-</sup> encephalopathy [6]                     | oral, 0.5, 8.0, 20 mg/kg                               | 0.5 no effect<br>8.0 increased<br>20 increased | Mitochondria improved (EM) | Improved       | Not reported/unclear             |
| <i>Tk2-H126N</i> knock-in encephalopathy, myopathy [7]              | oral to dam, 0.8 mg/kg (pregnancy) 4 mg/kg (postnatal) | Increased                                      | Unaffected                 | Unaffected     | Unaffected/unaffected            |
| Skeletal muscle-specific <i>Cox15</i> <sup>-/-</sup> , myopathy [8] | i.p., 8.0 mg/kg daily                                  | Not reported                                   | Improved                   | Improved       | Unaffected/decreased             |
| <i>Coq9</i> <sup>p.R239X</sup> encephalopathy [2]                   | oral, 28 or 225 ppm in chow                            | 28 no effect<br>225 decreased                  | Slight improvement         | Unaffected     | Unaffected/decreased (high dose) |

dose. Activation of autophagy, possibly resulting in improved clearance of damaged cellular components and their recycling for fuel has been suggested as a therapeutic mechanism for rapamycin. Clearly, better understanding of the role of autophagy in the pathogenesis of mitochondrial diseases, as well as of the effect of rapamycin on it, is needed. Moreover, the reliability of the methods to assess autophagy in autopsy samples is still lacking.

This study is interesting and important firstly because there is a chronic need to see more well-conducted preclinical trials with negative outcomes published. This will fight publication bias that is obviously common in both preclinical and clinical research. Secondly, it underscores that therapeutic targeting of a single pathway, no matter how central physiologically and how successful in other models, is unlikely to work for all mitochondrial disorders. The metabolic derangements in each disorder must first be characterized in detail and thereafter a focused strategy designed and investigated in sufficiently large experimental groups to enable recording of both beneficial and adverse events. There will likely not be one drug for the vast spectrum of different mitochondrial diseases [10]. Additionally, the drug doses used in mouse studies raise the question of safety, as they tend to be higher than those approved for humans.

In summary, it is of utmost importance to publish also the preclinical studies showing no benefit of a proposed new treatment. Moreover, the experimental animal groups should be large enough to show biologically meaningful, statistically robust results. It remains an unanswered question how many trials showing no benefit of a drug or other intervention have been performed and left unpublished. To ensure accumulation and availability of unbiased evidence we suggest that preclinical animal studies using drugs already approved for human use – and thus easier to adopt to clinical trials than novel molecules – should be

mandatorily registered in a preclinical database, as is the case for clinical trials.

## Disclosure

The authors declared no conflicts of interest.

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