The Identification of Caffeine Analogs as Putative TORC1 Inhibitors Using a Drug-Sensitive Yeast Model

Judd Williams, OMS-II, Anna Breen, B.S., Brian M. Wasko, PhD
Western University of Health Sciences

Western University of Health Sciences
College of Osteopathic Medicine of the Pacific
COMP-Northwest

Background

The mechanistic target of rapamycin (mTOR or TOR) is a protein kinase that serves as a central regulator of cell growth and metabolism. Inhibition of mTOR both genetically and pharmacologically (via rapamycin) has been found to increase the lifespan of diverse species from yeast to mice. Rapamycin is clinically approved for use as an immunosuppressant. Rapamycin mechanistically rapamycin inhibits the mTORC1 complex after binding and forming a complex with the protein FKBP12 (Fpr1). Our laboratory has generated a series of TOR pathway mutations in a yeast strain background that lacks multiple drug efflux pumps. The growth profiles of the strains in the presence of drugs are used to identify TOR inhibitors. Caffeine has previously been identified to have TOR1 dependent effects on yeast growth, and to impact the mTOR pathway in mammalian cells. We have found that mutants deficient in TOR1 function display growth sensitivity to theophylline and aminophylline, analogs of caffeine. Control yeast strains with intact drug efflux systems were resistant to these compounds, suggesting the compounds are actively effluxed from wildtype yeast cells.

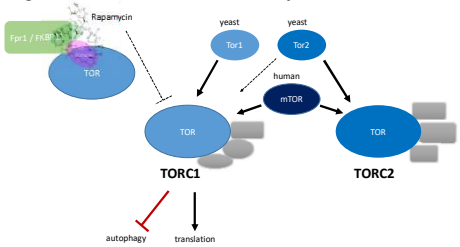
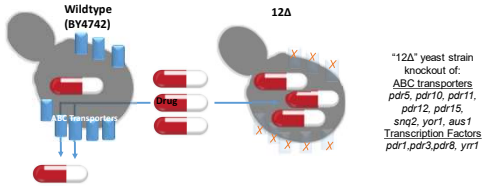


Figure 1. Schematic of TOR complex 1 and complex 2.

Methodology



strain annotation	type of mutation	TOR related phenotype	reference
tor1	loss of function	sensitivity to TORC1 inhibitors	Heitman et al., Lee et al.
fpr1	loss of function	resistant to rapamycin	Heitman et al., Lee et al.
tor1-S1972R	missence	resistant to rapamycin	Heitman et al.
tor1-I1954V	missence	resistance to caffeine	Reinke et al.

Figure 2. Comparison of drug resistant (wildtype) and drug sensitive (12Δ) strains of yeast. BY4742 is a commonly used wildtype laboratory strain and 12Δ lacks 12 genes involved in plasma membrane drug efflux systems (Chinen et al).

Results

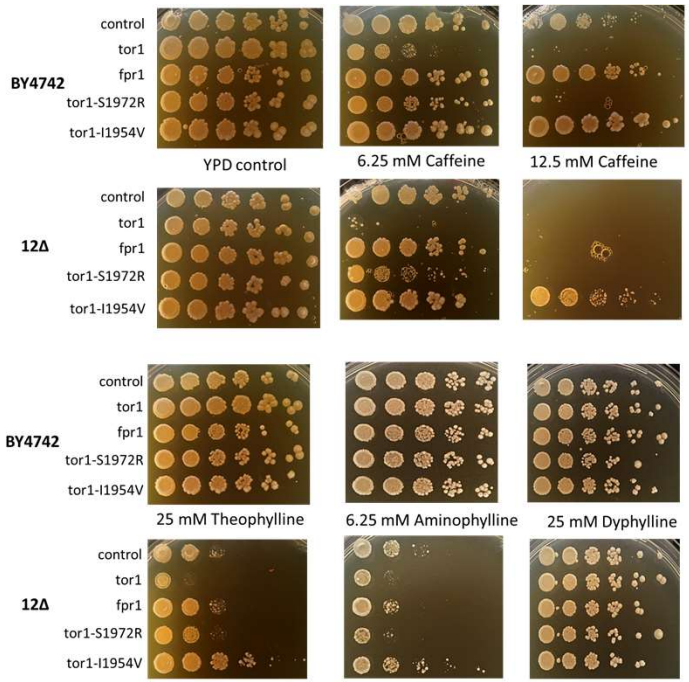


Figure 3. Theophylline and aminophylline inhibit growth of drug sensitive 12Δ yeast in a TOR pathway mediated manner**.** Growth assays of yeast strains serially diluted 1/5 and plating onto agar containing YPD media.

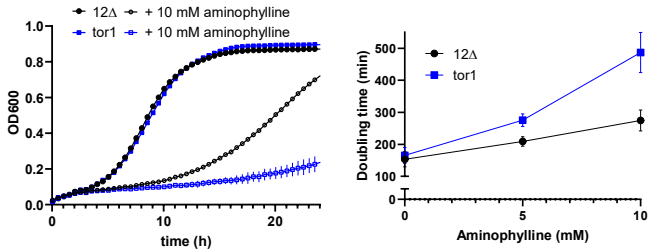


Figure 4. Liquid growth assays also quantitatively indicate that tor1 is sensitive to aminophylline in the 12Δ background in a concentration dependent manner.

Discussion

- The 12Δ strain exhibits increased sensitivity to caffeine theophylline, and aminophylline compared to the wildtype control strain. This suggests that these compounds may be actively pumped out of yeast cells via plasma membrane ABC transporters.
- Loss of tor1 renders cells more sensitive to growth impairment by theophylline and aminophylline, suggesting the growth inhibition is mediated by TORC1.
- The 12Δ strain was required to identify that theophylline displays enhanced growth inhibition in the absence of tor1, providing a proof-of-principle that the drug sensitive strain can identify putative TORC1 inhibitors that the wildtype control strain background would not identify.
- Dyphylline did not impair growth when tested at 25 mM in wildtype or 12Δ cells, suggesting its substituent at N7 results in a loss of growth inhibition potentially due to a lack of effects on the TOR pathway or poor cellular uptake.
- A literature search revealed that theophylline has been found to inhibit the mTOR pathway in the 3T3-L1 mouse adipocyte cell line and blocks mTOR activity in vitro (Scott and Lawrence. 1998), and our data further support a direct inhibitory effect on TOR complex I.

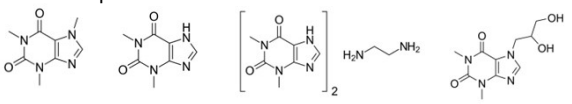


Figure 5. Drugs used in this study.

References

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