Characterization of Small Molecule Inhibitors of Drp1

Ashley Mallat[1], Ross Fredenburg[1], Samantha Lewis[2], Lauren Uchiyama[2], Jodi Nunnari[2], Nan Ji[1], Eric Bell[1], Yoh Terada[3], and Claire C. Tseng[1]

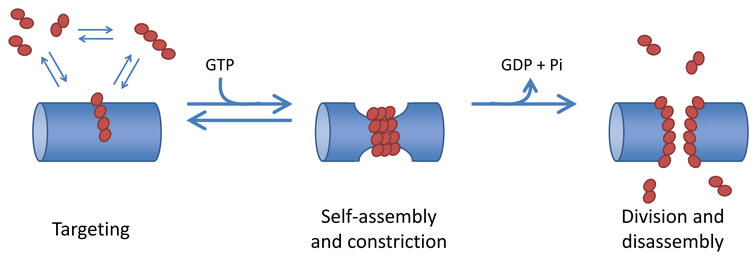
[1]Mitobridge, Inc., Cambridge MA; [2]University of California, Davis, Davis CA; [3]Drug Discovery Research, Astellas Pharma, Japan

Abstract

Mitochondria exist in a range of phenotypes from elongated tubes to small rounded fragments, with these phenotypes thought to reflect mitochondrial health. The fragmented phenotype is a typical response to numerous cellular stressors. Drp1 is the essential catalytic component of the mitochondrial fission machinery, physically constricting mitochondria at the point of fission. It is a potential therapeutic target for strategies aimed at improving the health of the mitochondrial network. Here we describe two small molecule inhibitors of Drp1, MB-0109 and MB-0223, that selectively and differentially inhibit oligomeric Drp1 enzymatic activity compared with that of other dynamin family members, Opa1 and dynamin-1 (Dnm1). In addition to a preference for the oligomeric form of Drp1 biochemically, they reverse the mtDNA defect associated with deletion of *mfn1*, a key mediator of mitochondrial outer membrane fusion. Finally, the compounds delay staurosporine-mediated release of cytochrome c, in which Drp1 plays a key role, and is a prelude to apoptosis. These compounds may be useful in elucidating the role of Drp1 and mitochondrial fission in response to stress.

Introduction

Figure 1: Schematic representation of Drp1 role in mitochondrial fission



Biochemical Assay Method

Figure 2: Rate of Drp1 GTPase activity is enhanced in the presence of liposomes

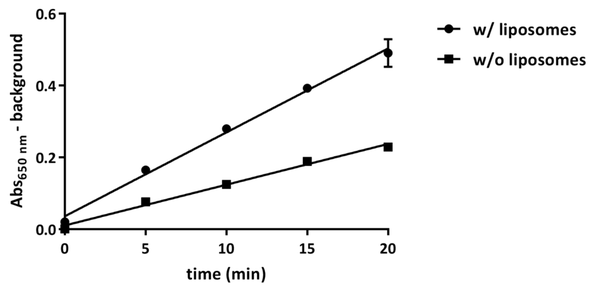
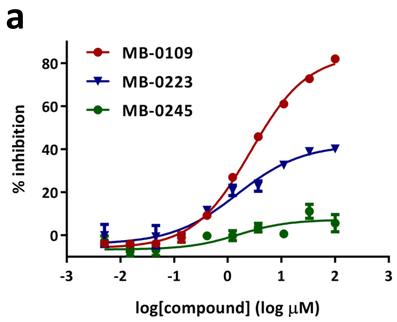


Figure 2: Drp1 GTPase activity was measured via malachite green detection of phosphate production by recombinant Drp1 in the presence or absence of liposomes containing 20% cardiolipin. Opa1 and Dnm1 GTPase activities were measured similarly.

Results

Figure 3: Active compounds selectively inhibit Drp1 GTPase activity

a



b

	IC50s (μM)			Max % inhibition
	Drp1	Opa1	Dnm1	
MB-0109	1.6	>100	>100	84
MB-0223	1.3	>100	>100	42
MB-0245	>100	>100	>100	5.7

c

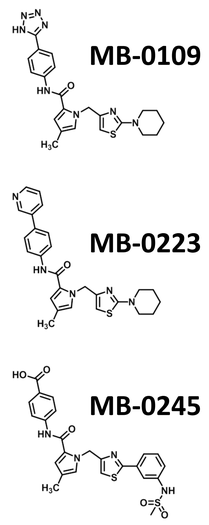


Figure 3: (a) IC_{50} curves in the presence of liposomes with 3 compounds from the same chemical scaffold. MB-0109 is a full inhibitor, with a max inhibition of 91% Drp1 activity. MB-0223 is a partial inhibitor, with a maximum inhibition of 67% of Drp1 activity. MB-0245 does not inhibit Drp1, and was used as a negative control. **(b)** MB-0109 and MB-0223 have low μM IC_{50} values, with >100-fold selectivity for Drp1 over two other dynamin-like GTPases, Opa1 and Dnm1. **(c)** Chemical structures of MB-0109, MB-0223, and MB-0245.

Figure 4: Selective inhibitors display liposome-dependent potency

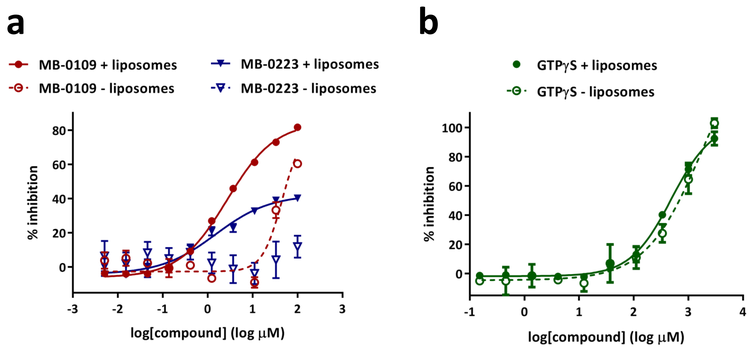


Figure 4: (a) The potencies of MB-0109 and MB-0223 are decreased in the absence of liposomes. Similar effects were seen with other full and partial inhibitors (not shown). **(b)** Drp1 inhibition via GTPγS (a non-hydrolyzable GTP analog) is not affected by the absence of liposomes.

Results

Figure 5: Rescue of mtDNA copy number defect in *mfn1-/-* MEFs

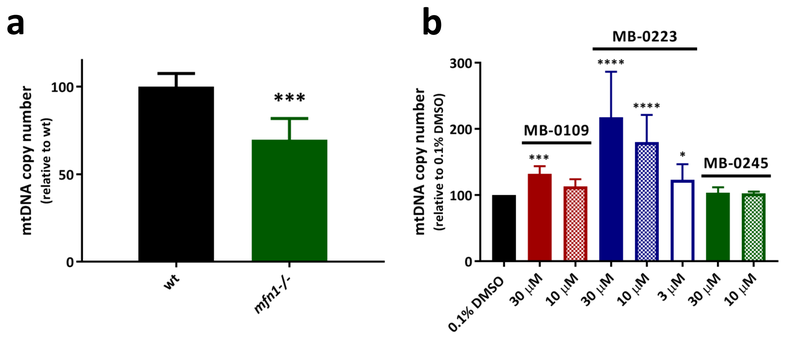


Figure 5: (a) mtDNA copy number is lower in *mfn1-/-* MEFs than in WT MEFs. **(b)** *mfn1-/-* MEFs were treated with compound for 48 hr before DNA isolation using DNeasy Blood and Tissue Kit (Qiagen). mtDNA copy number was assessed via qPCR, and normalized to nDNA. There is a dose-dependent increase in mtDNA copy number vs. 0.1% DMSO with MB-0109 and MB-0223. **** $p < 0.0001$, *** $p < 0.001$, * $p < 0.05$

Figure 6: Protection from staurosporine-induced cytochrome c release

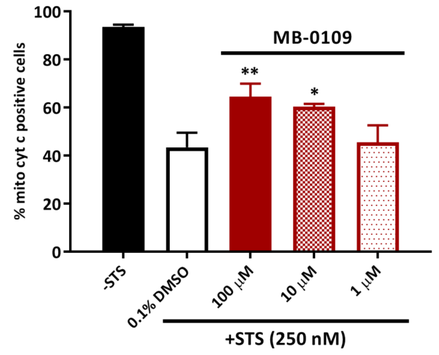


Figure 6: HeLa cells were pretreated with compound for 1 hr, and then co-treated with compound and staurosporine (STS) for 3 hr. Mitochondrial cytochrome c was measured via flow cytometry, using the FlowCellect Cytochrome c Kit (EMD Millipore). There is a dose-dependent increase in mitochondrial cytochrome c vs. 0.1% DMSO with MB-0109. ** $p < 0.01$, * $p < 0.05$

Conclusions and Future Directions

We have developed and characterized full and partial selective Drp1 inhibitors. We will use these compounds to better understand the complex mitochondrial fission machinery, with the goal to develop innovative therapeutics that improve mitochondrial function.

Acknowledgements

We thank our project team colleagues at Syngene (Hyderabad, India) for their efforts in the biochemical and cellular screening assays.

mitobridge