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To mdivi-1 or not to mdivi-1: Is that the question?

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Abstract

The fission/division and fusion of mitochondria are fundamental aspects of mitochondrial biology. The balance of fission and fusion sets the length of mitochondria in cells to serve their physiological requirements. The fission of mitochondria is markedly induced in many disease states and in response to cellular injury, resulting in the fragmentation of mitochondria into dysfunctional units. The mechanism that drives fission is dependent on the dynamin related protein 1 (Drp1) GTPase. mdivi-1 is a quinazolinone originally described as a selective inhibitor of Drp1, over other dynamin family members, and reported to inhibit mitochondrial fission. A recent study has challenged the activity of mdivi-1 as an inhibitor of Drp1. This study raises serious issues regarding the interpretation of data addressing the effects of mdivi-1 as reflective of the inhibition of Drp1 and thus fission. This commentary considers the evidence for and against mdivi-1 as an inhibitor of Drp1 and presents the following considerations; (1) the activity of mdivi-1 toward Drp1 GTPase activity requires further biochemical investigation, (2) as there is a large body of literature using mdivi-1 in vitro with effects as predicted for inhibition of Drp1 and mitochondrial fission, reviewed herein, the evidence is in favor of mdivi-1's originally described bioactivity, and (3) until the issue is resolved, experimental interpretations for the effects of mdivi-1 on inhibition of fission in cell and tissue experiments warrants stringent positive controls directly addressing the effects of mdivi-1 on fission.

Keywords

mitochondria; fission; fusion; division

INTRODUCTION

Mitochondria are dynamic organelles that undergo fission/division, fusion and intracellular transport. Mitochondria have multiple physiological functions including the generation of ATP through oxidative phosphorylation, the buffering of cytosolic calcium and the generation of reactive oxygen species. In normal healthy cells, fission and fusion are balanced to maintain mitochondria within length ranges appropriate for the maintenance of cellular physiology (Flippo and Strack, 2017). In contrast, in disease states or in response to injury, mitochondria undergo fragmentation into small dysfunctional units that in turn

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generate excessive amounts of reactive oxygen species leading to adverse cellular effects (Reddy et al., 2011; Balog et al., 2016; Golpich et al., 2017; Wu et al., 2017). The fission of mitochondria is mediated by the Drp1 GTPase. Drp1 is recruited to the mitochondrial outer membrane by a variety of adaptor proteins and then aggregates at the site of future fission along the mitochondrion (Hatch et al., 2014; Flippo and Strack, 2017). Drp1 forms oligomers that are considered to generate a ring around the mitochondrion that upon contraction serves to divide the mitochondrion into two separate mitochondria. There are several basic approaches to directly impair Drp1 function in cells; these include sh/siRNA mediated depletion of Drp1, genetic knock out of Drp1, the expression of dominant negative Drp1, treatment with cell permeable Drp1 targeting peptides and treatment with the pharmacological inhibitor mdivi-1. Pharmacological inhibitors can be powerful tools to control cellular processes because they allow for a greater degree of spatial and temporal control that manipulations that require prolonged time periods and impact the cell as a whole (e.g., depletion or overexpression studies). Given the morphological complexity of neurons and the reliance of neuronal function on specialized subcellular structures such as growth cones and synapses, a pharmacological tool to address the role of mitochondrial fission with high spatio-temporal resolution would be of very useful to the field. For example, a pharmacological inhibitor of fission could be applied with high spatio-temporal control to subsets of synapses or the growth cone through localized microperfusion approaches.

mdivi-1 is a cell permeable quinazolinone originally described by Cassidy-Stone et al (2008) as an inhibitor of Drp1 function in yeast and mammalian cells through biochemical and functional analysis, respectively. mdivi-1 was discovered through screening a library of 23,100 compounds for effects on yeast growth (primary screen) with subsequent screening of hits on mitochondrial morphology (secondary screen). The dual-screen resulted in a total of 9 related compounds with high (2), moderate (3) and low (4) activity in the secondary screen. Biochemical analysis revealed that mdivi-1 exhibits a Ki of 1–50 μM toward the GTPase activity of yeast Drp1. However, mdivi-1 did not affect the GTPase activity of the isolated generic Drp GTPase domain indicating that its impact on GTPase activity is not through direct effects on the GTPase domain. The authors were not successful in determining the activity toward mammalian Drp1 due to technical issues related to mammalian Drp1 preparations. Based on additional data showing that mdivi-1 prevents Drp1 self-assembly into rings and its association with mitochondria, Cassidy-Stone et al (2008) concluded that mdivi-1 likely impairs Drp1 function by acting allosterically and preventing Drp1 oligomerization which is in turn required for GTPase activity. mdivi-1 has since been used in 126 primary research publications (PubMed search for the term “mdivi”, July 2017).

The ability of mdivi-1 to inhibit Drp1 and impact mitochondrial fission has recently been challenged by Bordt et al (2017). In this report the authors did not find any effects of mdivi-1 treatment on mitochondrial morphology in mammalian cells (primary neurons and COS-7 cells) and obtained a Ki of >1.2 mM through biochemical analysis of mammalian Drp1 GTPase activity, although they confirmed the results of Cassidy-Stone et al (2008) of the activity of mdivi-1 toward yeast Drp1. Furthermore, Bordt et al (2017) report that mdivi-1 inhibits complex I of the electron transport chain through a not fully elucidated mechanism. The effects of mdivi-1 reported by Bordt et al (2017) on complex I were

observed at concentrations greater than 25 μM in primary neurons (50 μM minimal effective dose reported) and equal to and greater than 25 μM in COS-7 cells (with 25 μM giving only a partial effect).

This article considers the available literature using midiv-1 and asks whether the published evidence is in favor of or against the efficacy of mdivi-1 as an inhibitor of mitochondrial fission. Herein, focus is placed on in vitro studies in which the concentration of mdivi-1 is known and its effects on mitochondrial morphology have been directly determined (for consideration of studies using mdivi-1 in vivo see the review by Rosdah et al., 2016).

Consideration of the efficacy of mdivi-1 in preventing mitochondria fission in mammalian cells in vitro

In the studies addressing the effects of treatment with mdivi-1 on COS-7 and primary neurons Bordt et al (2017) report no effect on steady state mitochondrial morphology, even with prolonged drug treatments at concentrations of 50–100 μM. This is in contrast to the initial report by Cassidy-Stone et al (2008) in which the same concentration range of mdivi-1 promoted the formation of mitochondrial networks in COS-7 cells and prevented staurosporine induced mitochondrial fission in COS-7 cells, the latter an effect that Bordt et al (2017) were also not able to replicate.

In order to gain insights into the reproducibility of the general observations regarding the cellular bioactivity of mdivi-1 reported by the Cassidy-Stone et al (2008) and Bordt et al (2017) we can turn our attention to other reports using mdivi-1. A PubMed search was performed using mdivi as the search term and studies containing in vitro data (excluding Cassidy-Stone et al (2008) and Bordt et al (2017)) were compiled (Table I). Papers that directly monitored mitochondrial morphology were then considered to determine the relationship between mdivi-1 treatment and its effect on mitochondrial morphology (n=42). Of these papers, 12/42 (29%) used rat cells, 20/42 (48%) used human cells, 11/42 (26%) used mouse cells and one used a grouper cell line. 40/42 (95%) of papers reported effects of mdivi-1 on cells consistent with its initially reported action as an inhibitor of Drp1 function as determined by analysis of mitochondrial network morphology or mitochondrial length and/or number (Table I). Of these, 11/40 (28%) also provided evidence that Drp1 depletion (labeled Dp in Table I) and/or expression of dominant negative Drp1 (labeled DN in Table I) had effects consistent with mdivi-1. Furthermore, 28/40 (70%) of the studies showing altered mitochondrial morphology with mdivi-1 also provided additional supporting evidence that Drp1 either exhibited increased recruitment to mitochondria, and/or that Drp1 underwent phosphorylation-mediated activation, and/or increased levels of Drp1 expression (Table I, labeled E+). 32/40 (80%) of the studies presented either Sh/DN or E+ data to support a role for Drp1 mediated fission in conjunction with the results of mdivi-1 treatment. 35/40 (88%) of studies used mdivi-1 at concentrations equal to or less than 25 μM (Table I). Bordt et al (2017) observed either no effect or a low partial effect on complex I at 25 μM mdivi-1 depending on cell type, indicating the effect of mdivi-1 on complex I is not likely to have contributed in studies using equal to or less than 25 μM.

2/42 (5%) of papers did not find effects of mdivi-1 on mitochondria shortening/fission (Table I). Interestingly, one of these two papers (Suzuki-Karasaki et al, 2015) unexpectedly found that both mdivi-1 and Drp1 depletion decreased mitochondrial lengths, showing consistency between the two treatments albeit in the opposite direction as expected for the involvement of Drp1 in fission. This may reflect a context dependent role of Cdk5 phosphorylated Drp1 that has also been reported to impair instead of promote fission (Cho et al., 2014). Thus, although having effects opposite to that expected on mitochondrial morphology, Suzuki-Karasaki et al (2015) do report consistency between the two manipulations impacting Drp1 function in a similar manner. The other paper reporting no effect of mdivi-1 (Cunniff et al., 2013) found no effect on nitroxide induced changes in the cellular distribution and morphology of mitochondria, which may or may not reflect fission. However, Cunniff et al (2013) also report that treatment with mdivi-1 alone resulted in effects on mitochondria morphology consistent with inhibition of fission but do not specifically provide the data and analysis (hence this report was parsimoniously considered as belonging to the no effect of mdivi-1 category).

As originally discussed in the Cassidy-Stone et al (2008) paper mdivi-1 preparations contain two atropisomers of mdivi-1, although whether they may alter in bioactivity is unknown. This raises the possibility that the commercial source of mdivi-1 may contain different ratios of the atropisomers, or other differences in the quality of the preparation. However, the commercial source of mdivi-1 most likely does not explain the differences in the reported effects of mdivi-1. 11/40 papers did not report the source of mdivi-1, 17/40 obtained it from Sigma, 7/40 obtained it from Enzo, 2/40 from Santa Cruz and 1 from Bionet, Tocris and Key Organics each. Suzuki-Karasaki et al (2015) report no effect of mdivi-1 on preventing mitochondria fragmentation or increasing steady state lengths and obtained mdivi-1 from Enzo. However, 6 other papers reporting mdivi-1 effects consistent with Drp1 inhibition also obtained mdivi-1 from Enzo, with one reporting consistency between the effects of mdivi-1 and Drp1 depletion. Thus, there is no apparent correlation between the effects of mdivi-1 and its source. The paper by Bordt et al (2017) obtained mdivi-1 from Sigma and Enzo, both sources that as noted above have provided mdivi-1 for studies that consistently report effects of mdivi-1, arguing against the source as a variable in the discrepancy between the results in Bordt et al (2017) and the majority of the literature.

An additional parameter that might impact the efficacy of mdivi-1 is the drug's solubility. mdivi-1 is cell permeable and hydrophobic, and DMSO is the recommended solvent. Ten of the reports under consideration provided information regarding the stock concentration used to attain the final concentrations in vitro (denoted in Table I by [S]), or contained sufficient information to derive the stock concentration. The reported stock concentrations, all in DMSO, ranged from 2–283 mM. Six used 50 mM stocks, one used a 60 mM stock, one used a 5 mM stock, one used a 2 mM stock and one used a 283 mM stock. Cassidy-Stone et al (2008) used stock concentrations of 28 mM. Bordt et al (2017) did not report the stock concentration of mdivi-1. All papers reporting the stock concentration fell in the category finding effects of mdivi-1 consistent with its activity toward Drp1. The two papers discussed above that did not report clear effects of mdivi-1 on change in mitochondria morphology did not report stock concentrations. The final concentrations of DMSO in experiments involving treatment with mdivi-1 ranged from 0.02–0.25% DMSO. Thus, stock concentrations of

mdivi-1 in DMSO within the 5–60 mM range appear to be well suited to obtain effects when used in vitro with final DMSO up to 0.25%. In addition, as with any drug, storage conditions and freeze-thaw cycles should also be taken into consideration. It is not customary to report such parameters and indeed none of the papers under consideration provided this information. However, it would be cautious to aliquot the stock into volumes stored at −20° to −80° intended to be used only once and not subjected to freeze-thaw cycles. Whether such parameters may have contributed to the discrepancy between Bordt et al (2017) and the rest of literature cannot be evaluated based on the available information.

This analysis of the literature indicates that there are numerous studies using cells in vitro reporting effects of mdivi-1 treatment at concentrations below 25 μM consistent with effects on Drp1-mediated fission. Many of the reports also addressed the issue through molecular manipulation of Drp1 or analysis of Drp1 activity and showed results consistent with the effects of mdivi-1 impacting fission. Thus, the weight of the available evidence obtained from the use of mdivi-1 in cultured cells is in favor of mdivi-1 exhibiting inhibitory effects on mitochondrial fission in mammalian cells and consistent with targeting Drp1. mdivi-1 has also been used extensively for in vivo treatments but in these cases the concentration in tissues is not known and these studies are thus not specifically considered herein. However, a cohort of these in vivo studies present analysis of mitochondrial morphology and length in tissues and report effects of mdivi-1 consistent with its initial description as a Drp1 inhibitor (see the review by Rosdah et al., 2016).

Biochemical analysis of mdivi-1 as an inhibitor of Drp1 GTPase activity

One of the major observations reported by Bordt et al (2017) is that mdivi-1 inhibits the GTPase activity of yeast but not mammalian Drp1. However, Numadate et al (2014) reported finding inhibition of mammalian Drp1 GTPase activity by mdivi-1 with a Ki of 13 μM, similar to that determined by Cassidy-Stone et al (2008) for yeast Drp1. I note that Numadate et al (2014) report obtaining the open reading frame cDNA for Drp1 from GeneCopoeia™ (www.genecopoeia.com) that only has human and mouse Drp1 in its catalog, but we were not able to obtain a response from the authors to verify the species (that is not reported). However, direct communication with a GeneCopoeia representative verified they do not carry the yeast Drp1 ORF cDNA. Whether Numadate used human or mouse Drp1 remains unclear. Regardless, while there is a growing consensus that mdivi-1 inhibits yeast Drp1 GTPase activity, the studies by Bordt et al (2017) and Numadate et al (2014) present opposing findings for the effects of mdivi-1 on mammalian GTPase activity. Clearly, additional investigation of the effects of mdivi-1 on mammalian Drp1 GTPase activity and oligomerization into rings is required to resolve the discrepancy.

Final considerations

The above review of the literature argues in favor of the originally described bioactivity of mdivi-1 as an inhibitor of Drp1 mediated fission in mammalian cells. However, the report by Bordt et al (2017) cautions against the use of mdivi-1 as the sole approach to investigate mitochondrial fission or Drp1 function in cells. Minimally, if mdivi-1 is the only approach available to investigators, then stringent positive controls ought to be performed to determine

the effects of mdivi-1 on fission. Mitochondrial number and length should both be determined as fission would coordinately increase and decrease these two variables, respectively, and mdivi-1 treatment ought to impact both if acting on the fission mechanism. Mitochondria can undergo rounding or swelling and these morphological changes may be misinterpreted as reflective of fission based on length or circularity measurements alone. Ideally, live imaging of fission and fusion rates would also be used to obtain the most direct data addressing the impact of mdivi-1 treatment on the balance between fission and fusion. Studies relying on mdivi-1 also ought to consider whether the levels of Drp1 activation and/or recruitment to the mitochondrial surface are impacted by whatever manipulation affects mitochondria length and number in an mdivi-1 sensitive manner. Additionally, as with any pharmacological inhibitor, dose responses should be provided. Furthermore, whenever possible Drp1 function should also be manipulated by either knocking down Drp1 or expressing dominant negative Drp1 to determine consistency between the effects of mdivi-1 treatment and alternative approaches to manipulate Drp1 function. These determinations are best suited for in vitro analysis under strictly controlled conditions, but electron microscopic analysis of mitochondria, or analysis of fluorescently labeled mitochondria, in the context of in vivo experiments would also greatly benefit any study using mdivi-1 in vivo.

Although a flag of caution has been raised by Bordt et al (2017) regarding the suitability of mdivi-1 as a tool to manipulate Drp1 function, consideration of the literature using mdivi-1 under controlled in vitro conditions does not support the notion that the field should stop using mdivi-1 to address mitochondrial fission. Rather, the field needs additional well controlled investigations of the effects of mdivi-1 on mitochondrial fission. As noted previously, the activity of mdivi-1 toward Drp1 ring formation and GTPase activity certainly warrants additional scrutiny. Furthermore, the effects of mdivi-1 on complex I also need to be taken into consideration and proper controls be applied to determine possible effects through impairment of complex I function. Consistent with the report by Bordt et al (2017), Qian et al (2014) also observed that treatment with 50 μM mdivi-1 resulted in an approximate 50% decrease in oxygen consumption by transformed MEF cells in a manner independent of Drp1. In contrast, under conditions of high glucose Huang et al (2015) report that 10 μM mdivi-1, Drp1 siRNA and dominant negative Drp1 all increased complex I activity in human neuronal SK cell line. These observation only serve to further increase caution as Drp1 may have context dependent functions, further compounded by the ability of some Drp1 isoforms to regulate aspects of microtubule dynamics (Strack et al., 2013).

In conclusion, the analysis of the literature provided in this commentary indicates that the current evidence is strongly in favor of mdivi-1 having the expected bioactivity toward fission, and thus likely Drp1 activity, in mammalian cells. A caveat is that negative results are often not reported and there may be an unpublished body of evidence countering the published evidence. Future investigations using mdivi-1 to inhibit fission should provide additional evidence for the involvement of Drp1 as outlined above (e.g., inclusion of data from experiments in the Dn, Dp and E+ categories considered herein and ideally direct imaging of whether fission underlies any observed effects of experimental manipulations on mitochondrial morphology).

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TABLE I

Summary of studies using mdivi-1 in vitro that also monitored mitochondrial morphology

Papers reporting effects of mdivi-1 effects on mitochondria morphology consistent with the impairment of fission			
Reference	**Cell type**	**Concentration**	**Reported effects on mitochondria**
1. Magalon et al (2016)	Rat oligodendrocytes	1 μM[S]	increased length, decreased number
2. Lim et al (2015)	Rat vascular SMC	20 μM	inhibited angiotensin II and H202 induced decrease in length (E+)
3. Kim et al (2016)	Human neuroblastoma	1 μM	increased length, inhibited Aβ induced decrease in length (E+)
4. Su et al (2014)	Grouper GF-1 cells	5 μM	inhibited β-nodavirus B2 protein induced decrease in length (E+)
5. Han et al (2015)	Human breast cancer cells	5 μM	inhibited hypoxia and CDDp induced decrease in length (Dp)
6. Sharp et al (2017)	Mouse cardiac myocytes	5 μM	inhibited ischemia-reperfusion induced decrease in length (Dp, E+)
7. Gan et al (2014)	Human SH-SY5Y hybrid cells	10 μM[S]	increased length (Dp, DN, E+)
8. Tanner et al (2016)	Human endothelial cells	10 μM	inhibited fragmentation induced by low glucose (Dp, E+)
9. Xu et al (2016)	Rat cortical neurons	10 μM	inhibited fragmentation induced by desflurane
10. Steketee et al (2012)	Rat retinal ganglion cells	20 μM	increased lengths
11. Cunniff et al (2014)	Human mesothelioma cells	10 μM	increased mitochondrial networks (E+)
12. Salabei and Hill (2013)	Rat SMC	10 μM	inhibited PDGF induced length decrease
13. Chen et al (2016)	Human endothelial cells	25 μM	inhibited angiotensin-II induced decrease in length
14. Twaroski et al (2015)	Human SCDN	25 μM[S]	inhibited anesthetic induced decrease in length (E+)
15. Gao et al (2016)	Mouse cardiomyocytes	50 μM[S]	inhibited H202 induced decrease in length (E+)
16. Yu et al (2016)	Rat cardiomyocytes	100 μM	increased mitochondria volume, decreased number (E+)
17. Hong et al (2013)	Human SMC	20 μM	inhibited fission induced by hyper-oxygenation (E+)
18. Vazquez-Martin et al (2012)	Mouse fibroblasts	50 μM	increased lengths
19. Alaimo et al (2014)	Rat astrocytoma cells	1 nM[a]	decreased manganese induced fragmentation (Dp)
20. Kim et al (2013)	Mouse C2C12 cells	10–20 μM[S]	increased length (E+)
21. Wan et al (2014)	Human glioblastoma	5 μM	decreased hypoxia induced fragmentation (E+)
22. Solesio et al (2012)	Human SH-SY5Y cells	10 μM	decreased fragmentation induced by 6-OHDA (E+)
23. Zhao et al (2014)	Human SH-SY5Y cells	10 μM[S]	inhibited oxygen-glucose deprivation induced fragmentation (Dp)
24. Zhang et al (2013)	Human epithelial cells	50 μM	inhibited irradiation induced fission (E+)[b]
25. Chlystum et al (2013)	Mouse fibroblasts	50 μM[S]	inhibited annexin-6 induced fission (E+)
26. Farrand et al (2013)	Human OVCA cells	50 μM	partial inhibition of cisplatin and piperlongumine induced fragmentation (E+)

Papers reporting effects of mdivi-1 effects on mitochondria morphology consistent with the impairment of fission			
Reference	**Cell type**	**Concentration**	**Reported effects on mitochondria**
27. Cui et al (2010)	Rat N27 cells	10 μM[S]	inhibited fragmentation in PINK1 siRNA treated cells and PINK1 I347P expressing cells (DN, E+)
28. Park et al (2013)	Mouse microglia	25 μM	inhibited LPS induced decrease in length (Dp, E+)
29. Liu et al (2013)	Mouse HT22 cells	5 μM	partial restoration of decreased length in RanBP9 expressing Cells
30. Maimaitijian et al (2016)	Rat SMC	10–25 μM	inhibited length decrease induced by high glucose (E+)
31. Zhuang et al (2017)	Rat SMC	10–25 μM	inhibited length decrease induced by high glucose (E+)
32. Liu et al (2017)	Rat hippocampal neurons	25 μM	inhibited length decrease induced by propofol
33. Zhou et al (2017a)	Mouse N2a cells	5 μM[S]	inhibited length decrease induced by oxygen-glucose deprivation/reperfusion (E+)
34. Zhou et al (2017b)	Human nasopharyngeal carcinoma	10–20 μM	inhibited length decrease induced by COX-2 expression (Dp, E+)
35. Zhang et al (2017)	Mouse MC3T3-β1 cells	10 μM[S]	inhibited length decrease induced by TNF-α (E+)
36. Huang et al (2015)	Human SK cells	10 μM	inhibited length decrease induced by high glucose (Dp, DN, E+)
37. Ong et al (2010)	Mouse HL-1 cardiomyocytes	10–50 μM	increased lengths (DN)
38. Chen et al (2016)	Human endothelial cells	25 μM	inhibited decreased length induced by angiotensin-II (E+)
39. Troncoso et al (2017)	Rat L6 myotubes	1 μM [*]	inhibited dexamethasone induced increase in mitochondria number and decrease in volume (E+)
40. Iqbal and Hood (2014)	Mouse C2C12 cells	25 μM	partially inhibited H202 induced fragmentation (E+)
Papers reporting a lack of mdivi-1 effects on mitochondria morphology			
Reference	**Cell type**	**Concentration**	**Reported effects on mitochondria**
41. Suzuki-Karasaki et al (2015)	Human melanoma cells	50 μM	no effect on Apo2L induced decrease in length
42. Cunniff et al (2013)	Human mesothelioma	10 μM	no effect on nitroxide induced fragmentation

SMC = smooth muscle cell

SCDN = stem cell derived neurons

Dp = mdivi-1 had same effect as Drp1 sh/siRNA

DN = mdivi-1 had same effect as expression of dominant negative Drp1

E+ = study presents additional evidence for Drp1 involvement in the ensuing mitochondrial changes

[a] this study found that in their system 1 mM mdivi-1 was toxic and nM concentrations were viable for the study

[b] this study also presented evidence that irradiation induced fission through live imaging

[S] as denoted in the concentration column this study provided sufficient information regarding the stock concentration

[*] obtained via Email exchange with the senior author