Neuroprotective Effects of Fosgonimeton on Dopaminergic Neurons Are Mediated by Signaling Effectors Downstream of HGF/MET

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CONCLUSIONS

1. Fosgo-AM, the active metabolite of fosgonimeton, improves neuronal survival, preserves neurite networks, and reduces α-syn aggregation in a cell culture model of dopaminergic neuron degeneration
2. Fosgo-AM exerts its neuroprotective effects through multiple kinases downstream of HGF/MET, including AKT, MEK/ERK, CaMKII, and PKC

KEY TAKEAWAY

Neuroprotective effects of fosgo-AM are driven by the activation of downstream effectors of HGF/MET signaling

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Disclosures

Sherif Reda, Robert Taylor, Jewel Johnston, and Kevin J. Church are all employees and stockholders of Athira Pharma, Inc.

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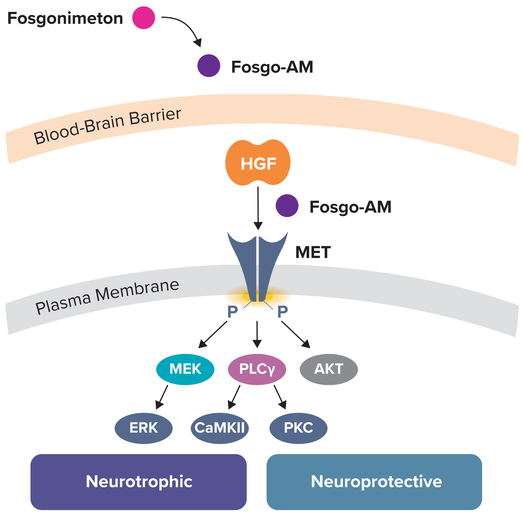
INTRODUCTION

Figure 1. Positive modulators of HGF/MET promote neuroprotective effects through downstream intracellular signaling pathways



- The binding of HGF to the MET receptor leads to the activation of several downstream signaling kinases, including AKT, MEK/ERK, CaMKII, and PKC[1]
- The HGF/MET system promotes cell survival, increases neuronal outgrowth, and modulates neural network repair through these signaling pathways[1-3]
- Fosgonimeton, which converts to its active metabolite fosgo-AM in the blood, is a small-molecule positive modulator of the HGF/MET system that has shown neurotrophic and neuroprotective effects in vitro and in vivo[4,5]
- MPP⁺ is a mitochondrial toxin that, when taken up by dopamine transporters in dopaminergic neurons, causes PD-relevant pathology in vitro and in vivo through interference with mitochondrial function that leads to ATP depletion, oxidative stress, and cell death[6,7]

OBJECTIVE

To determine the mechanisms by which fosgonimeton exerts its neuroprotective effects on dopaminergic neurons injured by administration of MPP⁺

METHODS

Primary culture of dopaminergic neurons

- Mesencephalic neurons from the midbrains of 15-day-old Wistar rat embryos were cultured in 96-well plates

Test compound and injury with mitochondrial toxin

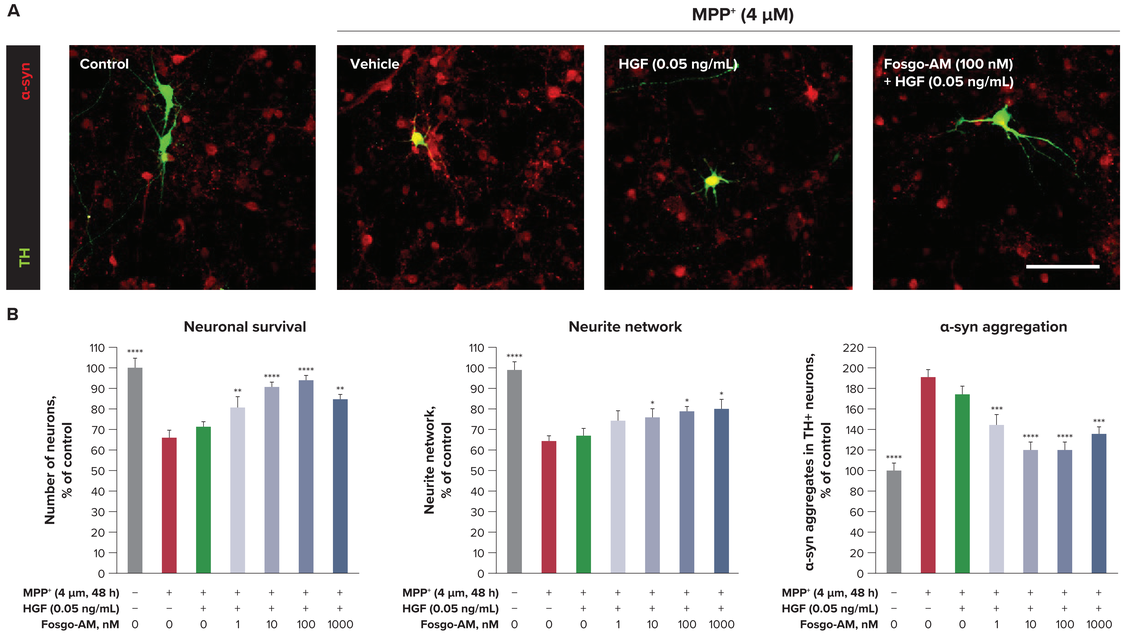
- On day 6 in culture, rat dopaminergic neurons were incubated with one of the following kinase inhibitors: GSK690693 (AKT inhibitor), PD98059 (MEK inhibitor), KN-62 (CaMKII inhibitor), or calphostin C (PKC inhibitor)
- 40 minutes after, neurons were treated with vehicle (DMSO, 0.1%) or HGF (0.05 ng/mL) plus fosgo-AM
- 20 minutes later, MPP⁺ was added to a final concentration of 4 µM for 48 hours in the presence of fosgo-AM and/or inhibitors

Immunostaining

- Co-immunostaining of TH and α-syn was performed to determine dopaminergic neuron survival (TH+ neurons), neurite network integrity (TH+ total neurite length in micrometers), and α-syn aggregation in TH+ dopaminergic neurons (overlapping TH and α-syn staining)

RESULTS

Figure 2. Fosgo-AM protects dopaminergic neurons, preserves neurite networks, and reduces α-syn aggregation in vitro

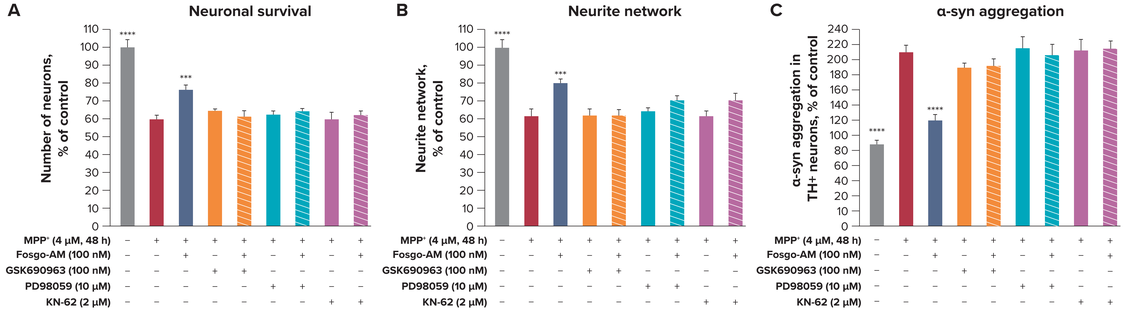


(A) Representative images of primary dopaminergic neurons under control conditions (left) with the MPP⁺ injury alone (center left), after MPP⁺ injury in the presence of subthreshold HGF (center right), and after MPP⁺ injury in the presence of fosgo-AM + subthreshold HGF (right). (B) Image quantification showed pretreatment with fosgo-AM + subthreshold HGF (blue bars) before MPP⁺ injury significantly increased the survival of dopaminergic neurons, protected the neurite network, and reduced the aggregation of α-syn compared with vehicle (red bars). Subthreshold HGF alone (green bars) did not have a significant effect on any of the three metrics. Culture medium contained subthreshold HGF (0.05 ng/mL). Scale bar: (A) 100 µm (all panels).

All data displayed as mean + SEM. One-way ANOVA followed by Fisher's LSD test. (Prism, version 9.5.0; GraphPad).

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

Figure 3. The neuroprotective effects of fosgo-AM are mediated by activation of AKT, MEK, and CaMKII



The presence of inhibitors of AKT (GSK690693, orange bars), MEK (PD98059, teal bars), or CaMKII (KN-62, pink bars) abolished the neuroprotective effects of fosgo-AM (blue bars) against MPP⁺ (red bars) on (A) neuronal survival, (B) preservation of neurite networks, and (C) α-syn aggregation in TH+ neurons when compared with control conditions (gray bars). Culture medium contained 0.05 ng/mL HGF.

All data displayed as mean + SEM. One-way ANOVA followed by Fisher's LSD test. (Prism, version 9.5.0; GraphPad).

***$p < 0.001$; ****$p < 0.0001$.

Abbreviations **AKT**, protein kinase B; **ANOVA**, analysis of variance; **α-syn**, α-synuclein; **ATP**, adenosine triphosphate; **CaMKII**, calmodulin-dependent protein kinase II; **DMSO**, dimethyl sulfoxide; **ERK**, extracellular signal-related kinase; **fosgo-AM,** active metabolite of fosgonimeton; **HGF**, hepatocyte growth factor; **LSD**, least significant difference; **MEK**, mitogen-activated protein kinase; **MPP⁺**, 1-methyl-4-phenylpyridium; **P**, phosphorylation; **PD**, Parkinson's disease; **PKC**, protein kinase C; **PLCγ**, phospholipase C γ; **SEM**, standard error of the mean; **TH**, tyrosine hydroxylase.

References **1.** Desole C et al. *Front Cell Dev Biol*. 2021;9;683609. **2.** Maina F et al. *Nat Neurosci*. 1999;2:213-217. **3.** Kitamura K et al. *Int J Mol*. 2019;20:20190228. **4.** Johnston JL et al. *Neurotherapeutics*. 2022;19:1413-1431. **5.** Reda S et al. Presented at AAIC; July 16-20, 2022; San Diego, CA, USA, and online. ePoster 65874. **6.** Dauer W, Przedborski S. *Neuron*. 2003;39:889-909. **7.** Javich JA et al. *Proc Natl Acad Sci U S A*. 1985;82:2173-2177.