Fosgonimeton, a Novel, Small-Molecule Positive Modulator of the HGF/MET System, Is Neuroprotective in Primary Neuron Culture

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CONCLUSIONS

1. Fosgo-AM, the active metabolite of fosgonimeton, increased the number of neurons in culture and enhanced neurite outgrowth
2. Cortical neurons treated with fosgo-AM protected against neurological insults that are central to neurodegeneration such as mitochondrial dysfunction, excitotoxicity, inflammation, and oxidative stress
3. The major signaling pathways of HGF (via MET) mediate the neuroprotective effect of fosgonimeton against glutamate excitotoxicity

KEY TAKEAWAY

Neuroprotective and neurotrophic effects of fosgo-AM highlight its therapeutic potential to restore neuronal health and slow or reverse neurodegeneration

Disclaimer

Fosgonimeton is an investigational drug product and is subject to ongoing clinical evaluation. It has not been approved by any regulatory authority for any commercial use.

Acknowledgments

This study was sponsored by Athira Pharma, Inc. Medical writing and editorial support was provided by Eileen McIver, PhD and Ashley Thoma, PharmD, of ApotheCom and funded by Athira Pharma, Inc.

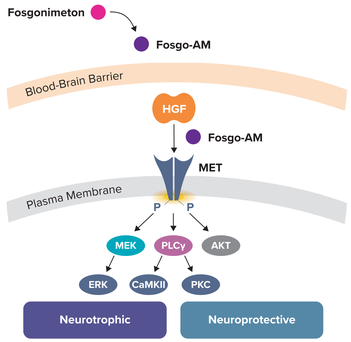
Disclosures

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

Presented at the 2023 ASENT Virtual Annual Meeting; March 13-15, 2023

INTRODUCTION

Figure 1. Fosgonimeton, a positive modulator of the HGF/MET pathway may counteract neurodegeneration through multiple mechanisms



- HGF signaling through the MET receptor promotes neurotrophic processes, such as neurite outgrowth, through several downstream signaling kinases[1]
- HGF/MET signaling also engages neuroprotective mechanisms that counteract several pathological hallmarks of neurodegeneration, such as excitotoxicity, neuroinflammation, and oxidative stress-induced damage[1-3]
- Positive modulators of HGF/MET may provide therapeutic benefit to patients with Alzheimer's disease, Parkinson's disease, ALS, multiple sclerosis, cerebral ischemia, and other injuries/degenerative diseases of the nervous system[1]
- Fosgonimeton is a small-molecule positive modulator of HGF/MET being evaluated for use in the treatment of Alzheimer's disease (LIFT-AD, NCT04488419), as well as for Parkinson's disease dementia or dementia with Lewy bodies (SHAPE, NCT04831281)
  - Fosgonimeton rapidly converts to a brain-penetrant active metabolite (fosgo-AM) after administration

OBJECTIVE

To explore the neurotrophic and neuroprotective effects of fosgo-AM on cultured rat primary cortical neurons

METHODS

Network growth assay

- Primary cortical neurons from rat embryos at embryonic day 15 were cultured for 2 days before testing
- A subthreshold concentration of HGF (0.01 ng/mL) with or without fosgo-AM (1 pM, 1 nM, or 100 nM) was added to neuronal cultures for 2 days
- Cells were fixed and immunostained with a mouse monoclonal antibody against MAP2 to reveal neuronal structures, including neurites, and were counterstained with Hoechst dye to reveal nuclei
- The number of neurons and total length of neurite networks were automatically quantified with ImageXpress acquisition and MetaXpress analysis software (Molecular Devices)

Neuroprotection assay

- Primary cortical neurons from rat pups on postnatal days 1-3 were cultured for 35-40 days before testing
- Neurons were treated with vehicle or fosgo-AM (1 nM, 10 nM, 100 nM, or 1000 nM) for 15 minutes, then given one of the following neurotoxic compounds: MPP$^+$, glutamate, LPS, or H_2O_2 for 24 hours
  - Fosgo-AM modulates the activity of the small amounts of HGF that are present in primary neuron cultures[4]
- The Cell Titer-Glo luminescent cell viability assay (Promega), which measures ATP levels, was used to calculate cell viability for each experimental group

MET occlusion assay

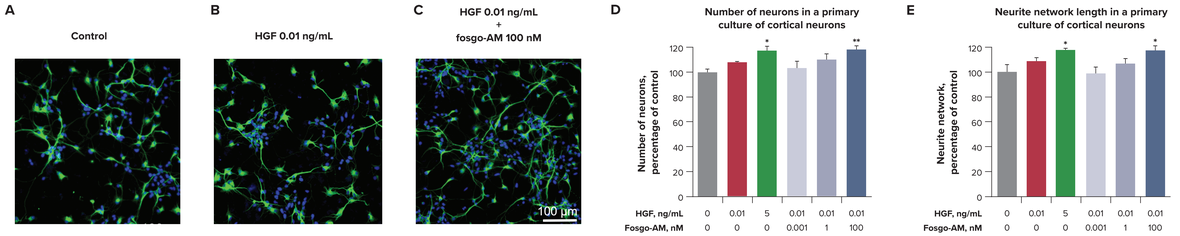
- Primary cortical neurons from rat embryos at embryonic day 15 were cultured for 12 days before testing
- Day 13 neuronal cultures were preincubated for 60 minutes with vehicle or one of the following inhibitors of MET-associated molecules: GSK690963 (100 nM; AKT inhibitor), calphostin C (200 nM; PKC inhibitor), KN-62 (2 µM; CaMKII inhibitor), or PD98059 (10 µM; MEK inhibitor)
- A subthreshold concentration of HGF (0.05 ng/mL), with or without fosgo-AM (100 nM), or an active concentration of HGF (5 ng/mL) was then added to neuronal cultures for the final 20 minutes of preincubation
- At the end of the preincubation period, a neurotoxic insult with glutamate (20 µM) was applied for 20 minutes, in the presence of fosgo-AM/HGF and inhibitors
- After the 20-minute injury with glutamate, test compounds and inhibitors were reintroduced for 24 hours
- Cells were fixed, immunostained, and quantified as for the network growth assay

Statistical analyses

- Prism 9 (GraphPad) 1-way ANOVA; post hoc Dunnett's multiple comparison test for network growth assays; and Tukey's multiple comparison test for cell viability assay
  - All data are displayed as mean + SEM
  - All *P* values are nominal and have not been corrected for multiple comparisons
- EnVision instrument software (PerkinElmer)

RESULTS

Figure 2. Fosgo-AM increased neuron numbers and enhanced neurite networks



Representative images from (**A**) control, (**B**) HGF 0.01 ng/mL, and (**C**) HGF 0.01 ng/mL + fosgo-AM 100 nM–treated primary cortical neuron cultures, with MAP2 visualized in green and nuclei stained blue (Hoechst dye). (**D**) Number of MAP2-positive neurons and (**E**) total length of neurite network present after a 48-hour incubation period.

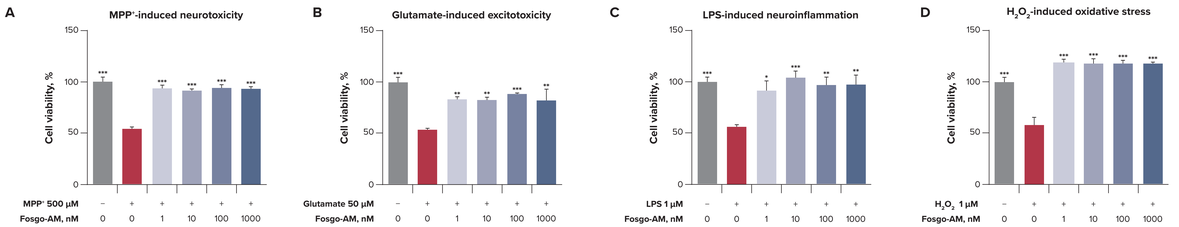
Data presented as mean + SEM. Scale bar in panel C applies to all images.

Statistical significance was determined by 1-way ANOVA with Dunnett's posttest.

*P < 0.05; **P < 0.01 compared with control (grey).

- Under basal conditions, the number of MAP2-positive neurons and the total length of the neurite network present after 48-hours incubation was significantly increased with 5 ng/mL HGF as well as with fosgo-AM (100 nM) + HGF (0.01 ng/mL), whereas HGF alone at 0.01 ng/mL did not have a significant effect
  - Fosgo-AM also augmented synaptic strength based on synaptobrevin-II staining intensity as an indication of the number of vesicles present at synapses (data not shown)

Figure 3. Fosgo-AM was neuroprotective against neurological insults



Percentage of cortical neurons surviving in culture after exposure to (**A**) MPP$^+$, (**B**) glutamate, (**C**) LPS, and (**D**) H_2O_2.

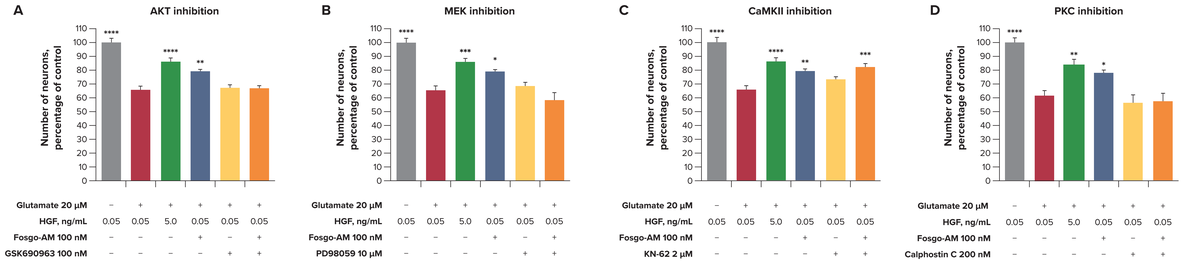
Data presented as mean + SEM.

Statistical significance was determined by 1-way ANOVA with Tukey's posttest.

*P < 0.05; **P < 0.01; ***P < 0.001 compared with insult alone (red).

- Treatment with fosgo-AM at all evaluated concentrations protected neurons from damage induced by neurotoxic insults that mimic several aspects of neurodegeneration: mitochondrial dysfunction (MPP$^+$), excitotoxicity (glutamate), neuroinflammation (LPS), and oxidative stress (H_2O_2)
- Treatment with 1 µM fosgo-AM rescued cell viability against neurological insults with the following percent recoveries: 84.9% with MPP$^+$, 61.6% with glutamate, 94.0% with LPS, and 144% with H_2O_2

Figure 4. Inhibition of the AKT, MEK, and PKC pathways downstream of HGF/MET signaling abolished the neuroprotective effects of fosgo-AM



Number of MAP2-positive neurons in culture following a 20-minute incubation in glutamate (20 µM) in the presence of (**A**) GSK690963, (**B**) PD98059, (**C**) KN-62, or (**D**) calphostin C. Data presented in panels A-C were generated from the same experiment (plate), data presented in panel D were generated from a separate experiment (plate).

Data presented as mean + SEM.

Statistical significance was determined by 1-way ANOVA with Tukey's posttest.

*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 compared with insult alone (red).

- Glutamate (20 µM for 20 minutes) induced a large and significant neuronal loss (neurons and neurite network). Fosgo-AM (100 nM) added 20 minutes before glutamate was able to significantly protect the neurons from the injury
- Neuroprotective effects of fosgo-AM were fully abolished by GSK690963 (AKT inhibition), PD98059 (MEK inhibition), KN-62 (CaMKII inhibition), and calphostin C (PKC inhibition)
- In the presence of KN-62 (CaMKII inhibition), fosgo-AM was still neuroprotective

Abbreviations **AKT**, protein kinase B; **ALS**, amyotrophic lateral sclerosis; **ANOVA**, analysis of variance; **ATP**, adenosine triphosphate; **CaMKII,** calcium/calmodulin-dependent protein kinase II; **ERK**, extracellular signal-related kinase; **fosgo-AM**, active metabolite of fosgonimeton; **H_2O_2**, hydrogen peroxide; **HGF**, hepatocyte growth factor; **LPS**, lipopolysaccharide; **MAP2**, microtubule-associated protein 2; **MEK**, mitogen-activated protein kinase kinase; **MPP$^+$**, 1-methyl-4-phenylpryidinium; **NMDA**, *N*-methyl-D-aspartate; **P**, phosphorylation; **PKC,** protein kinase C; **PLCγ**, phospholipase C γ; **ROS**, reactive oxygen species.

References **1.** Desole C et al. *Front Cell Dev Biol.* 2021;9:683609. **2.** Nicoleau C et al. *Stem Cells.* 2009;27:408-419. **3.** Ko KR et al. *Sci Rep.* 2018;8:8316. **4.** Gutierrez H et al. *Development.* 2004;131:3717-3726.