Fosgonimeton Promotes Survival and Reduces Protein Pathology in Primary Neuron Models of Alzheimer's and Parkinson's Disease

Kevin J. Church, Sherif Reda, Robert Taylor, Jewel Johnston

Athira Pharma, Inc., Bothell, WA, USA

CONCLUSIONS

1. Treatment with fosgo-AM significantly improved cortical neuron survival, protected neurite networks, and reduced p-Tau accumulation during injury with Aβ1-42 or glutamate
2. In dopaminergic neurons treated with fosgo-AM, there was a significant improvement in neuron survival, protection of neurite networks, and reduction of α-syn aggregation after injury with 6-OHDA or rotenone
3. These results show the potential of fosgonimeton to mitigate neuronal damage and protein pathology induced by mechanisms central to AD and PD

KEY TAKEAWAY

The broad neuroprotective effects of fosgonimeton (through fosgo-AM), in addition to its neurotrophic activity, highlight its ability to address multiple modes of neurodegeneration and its potential as a clinical candidate for AD and PD

Acknowledgments

This study was sponsored by Athira Pharma, Inc. Medical writing support was provided by Katie Henderson, PhD, of ApotheCom, and funded by Athira Pharma, Inc.

Disclosures

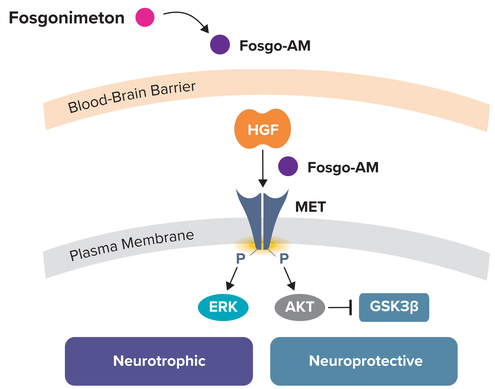
Kevin J. Church, Sherif Reda, Robert Taylor, and Jewel Johnston are employees and stockholders of Athira Pharma, Inc.

Presented at AD/PD™ 2023; March 28-April 1, 2023; Gothenburg, Sweden

INTRODUCTION

- Although prevalence of both AD and PD is increasing rapidly, treatment options are limited, highlighting the need for additional therapies[1,2]
- HGF, through its receptor MET, activates neuroprotective pathways, enhancing neurite outgrowth and survival of a variety of neurons, including cortical and dopaminergic neurons that degenerate in AD and PD, respectively[3]
- Fosgonimeton is a small-molecule positive modulator of the HGF/MET pathway that has shown promising neuroprotective and neurotrophic effects in preclinical studies that model AD and PD[4]
  - Clinical trials of fosgonimeton are currently enrolling people with AD[5,6]

Figure 1. Fosgonimeton positively modulates the neuroprotective and neurotrophic HGF/MET pathway



Fosgonimeton is converted in the blood to fosgo-AM, the active metabolite that crosses the blood-brain barrier and promotes HGF/MET-driven neurotrophic and neuroprotective signaling cascades in the brain.

OBJECTIVE

To evaluate the neuroprotective effects of fosgo-AM in cell culture models that capture components of neurodegeneration associated with AD and PD, including β-amyloid toxicity, p-Tau, α-syn aggregation, mitochondrial dysfunction, oxidative stress, and neuronal loss

METHODS

Figure 2. Experimental timeline

E15 brain dissection	Pretreatment + injury	Immunolabeling	Imaging
Cortex (AD models)	**Culture day 11** Step 1. Fosgo-AM (20 min) Step 2. Glutamate (20 min) or Aβ1-42 (15 μM, 24 h), with fosgo-AM Step 3. Fosgo-AM (24 h)	**MAP-2** (marker of neurons) + **AT100** (marker of p-Tau)	**Quantification** • Number of neurons • Neurite network • p-Tau
Ventral portion of the mesencephalic flexure (PD models)	**Culture day 6** Step 1. Fosgo-AM (20 min) Step 2. Rotenone (10 nM, 24 h) or 6-OHDA (20 μM, 24 h) with fosgo-AM	**TH** (marker of dopaminergic neurons) + **α-syn** (marker of protein aggregates)	**Quantification** • Number of neurons • Neurite network • α-syn aggregation

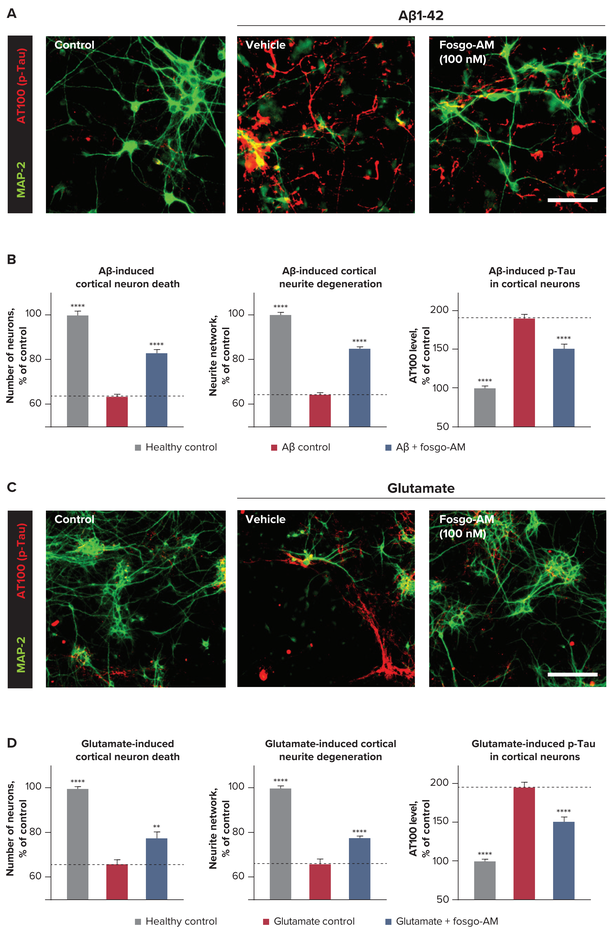
Primary cortical or dopaminergic neurons were treated with fosgo-AM and then subjected to various toxic injuries. Afterward, fosgo-AM treatment was reapplied for 24 hours. For Aβ1-42 and glutamate assays, culture medium was supplemented with HGF 0.05 ng/mL.

- The cortex or midbrain of each rat embryo was dissected at day E15, promptly placed in cold medium, and further dissected to isolate the appropriate brain region
- After injury, cells were fixed with 4% PFA, immunolabeled, imaged using ImageXpress (Molecular Devices), and analyzed using MetaXpress (Molecular Devices)

RESULTS

AD models

Figure 3. Fosgo-AM improves survival of cortical neurons and reduces tau hyperphosphorylation after exposure to Aβ1-42 or glutamate

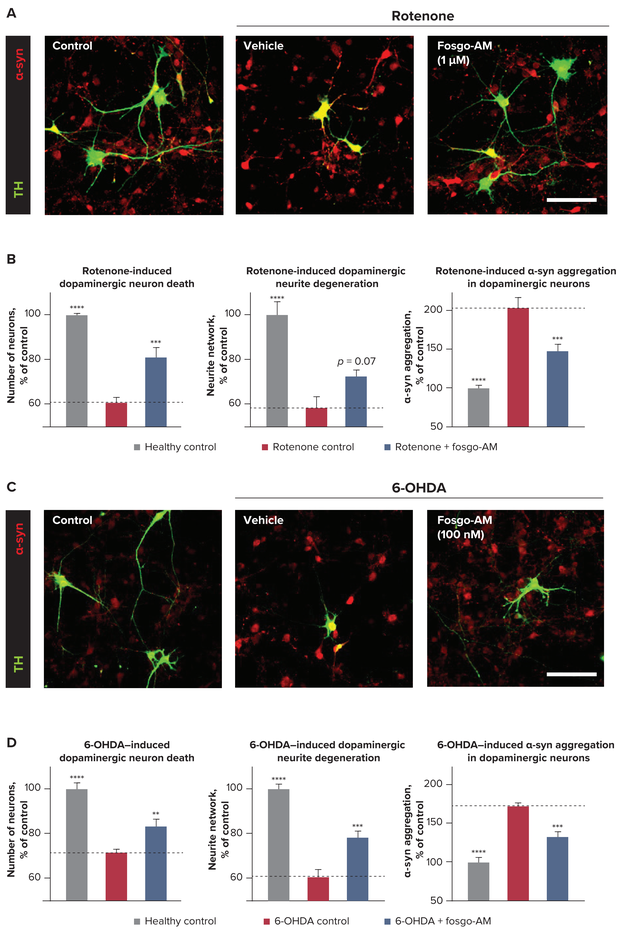


(A) Representative images from control primary cortical neuron cultures (left), cultures incubated with Aβ1-42 after treatment with negative vehicle control (center), and cultures incubated with Aβ1-42 after treatment with fosgo-AM (100 nM; right). Cultures were immunostained for MAP-2 (marker of neurons) and AT100 (marker for p-Tau). (B) Primary cortical neuron cultures that were pretreated with fosgo-AM had significantly higher numbers of neurons, longer neurite networks, and reduced p-Tau when subjected to Aβ1-42 (Fisher's LSD vs Aβ control [n = 5 or 6]). (C) Representative images from control cultures (left), cultures incubated with glutamate after treatment with negative vehicle control (center), and cultures incubated with glutamate after treatment with fosgo-AM (100 nM; right). Cultures were immunostained for MAP-2 and AT100. (D) Fosgo-AM (100 nM) significantly protected primary cortical neurons against injury with glutamate (Fisher's LSD vs glutamate control [n = 5 or 6]). Culture medium was supplemented with HGF 0.05 ng/mL. Scale bar: (A) and (C) 100 μm (all panels).

$^{**}p < 0.01$; $^{****}p < 0.0001$.

PD models

Figure 4. Fosgo-AM improves survival of dopaminergic neurons and reduces α-syn aggregation in cell culture models of PD



(A) Representative images from control primary dopaminergic neuron cultures (left), cultures incubated with rotenone after treatment with negative vehicle control (center), and cultures incubated with rotenone after treatment with fosgo-AM (1 μM; right). Cultures were immunostained with antibodies against TH (marker of dopaminergic neurons) and α-syn (marker of PD-associated protein aggregates). (B) Primary dopaminergic neuron cultures that were pretreated with fosgo-AM had significantly higher numbers of neurons (left), longer neurite networks (center), and reduced α-syn aggregation (right) when subjected to rotenone (Fisher's LSD vs rotenone control [n = 5 or 6]). (C) Representative images from control primary dopaminergic neuron cultures (left), cultures incubated with 6-OHDA after treatment with negative vehicle control (middle), and cultures incubated with 6-OHDA after treatment with fosgo-AM (100 nM; right). Cultures were immunostained with TH and α-syn. (D) Fosgo-AM (100 nM) significantly protected primary dopaminergic neurons against injury with 6-OHDA (Fisher's LSD vs 6-OHDA control [n = 4-6]). Scale bar: (A) and (C) 100 μm (all panels).

$^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

Abbreviations **6-OHDA**, 6-hydroxydopamine; **α-syn**, α-synuclein; **Aβ**, β-amyloid peptide; **AD**, Alzheimer's disease; **AKT**, protein kinase B; **E15**, embryonic day 15; **ERK**, extracellular signal-regulated kinase 1/2; **fosgo-AM**, active metabolite of fosgonimeton; **GSK3β**, glycogen synthase kinase-3 β; **HGF**, hepatocyte growth factor; **LSD**, least significant difference; **MAP-2**, microtubule associated protein 2; **P**, phosphorylation; **PD**, Parkinson's disease; **PFA**, paraformaldehyde; **p-Tau**, phosphorylated tau; **TH**, tyrosine hydroxylase.

References **1.** GBD 2016 Dementia Collaborators. *Lancet Neurol*. 2019;18:88-106. **2.** GBD 2016 Parkinson's Disease Collaborators. *Lancet Neurol*. 2018;17:939-953. **3.** Funakoshi H, Nakamura T. *Curr Signal Trans Ther*. 2011;6:156-167. **4.** Johnston JL et al. *Neurotherapeutics*. Published online December 20, 2022. doi: 10.1007/s13311-022-01325-5. **5.** ATH-1017 for Treatment of Mild to Moderate Alzheimer's Disease (LIFT-AD). ClinicalTrials.gov identifier: NCT04488419. Updated May 18, 2022. Accessed February 13, 2023. **6.** Open Label Study of ATH-1017 for Treatment of Mild to Moderate Alzheimer's Disease. ClinicalTrials.gov identifier: NCT04886063. Updated March 14, 2022. Accessed February 13, 2023.