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Fosgonimeton, a Small-Molecule Positive Modulator of the HGF/MET System, Attenuates Amyloid-β–Mediated Toxicity in Primary Neuron Cultures

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CONCLUSIONS

1. Treatment with fosgo-AM reduced tau phosphorylation and protected cortical neurons from degeneration induced by Aβ1-42
2. Neuroprotective effects of fosgo-AM are driven by its ability to promote prosurvival signaling cascades that counteract Aβ-mediated toxicity, including:
   - Protein pathology
   - Oxidative stress
   - Apoptotic signaling
   - Autophagy impairment

KEY TAKEAWAY

The neuroprotective effects of fosgonimeton, in addition to its neurotrophic and procognitive effects in preclinical models,[4] highlight its therapeutic potential to interrupt disease progression and restore neuronal health

Acknowledgments

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Disclosures

Sherif M. Reda, Wei Wu, Robert Taylor, Jewel Johnston, and Kevin J. Church are employees and stockholders of Athira Pharma, Inc. Leah Helton is a former employee and stockholder of Athira Pharma, Inc.

Disclaimer

Fosgonimeton is an investigational therapy that has not received FDA approval and has not been demonstrated to be safe or effective for any use.

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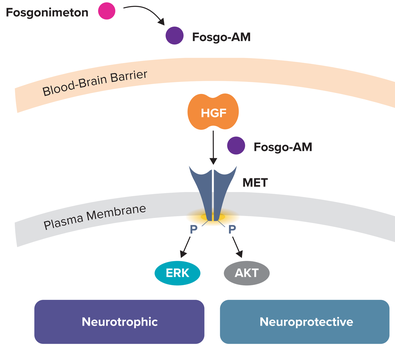
INTRODUCTION

- AD is a progressive and fatal neurodegenerative disorder with few therapeutic options that address the multimodal nature of the disease. Accumulation of abnormal proteins such as Aβ and pTau contributes to neurodegeneration[1]
- Positive modulation of the neurotrophic HGF/MET system may represent a promising therapeutic strategy for AD because of its multimodal neuroprotective and prosurvival effects that could address the complex pathophysiology of neurodegeneration[2,3]
- Downstream effectors of HGF/MET signaling, such as ERK and AKT, have been shown to activate prosurvival signaling cascades that counteract pathological components of AD, including excitotoxicity, mitochondrial dysfunction, oxidative stress, and protein aggregation[2,3]
- Fosgonimeton, a small-molecule positive modulator of the HGF/MET system, has shown neuroprotective, neurotrophic, and anti-inflammatory effects in preclinical models of dementia[4]



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

OBJECTIVE

To investigate the cellular mechanisms by which the active metabolite of fosgonimeton, fosgo-AM, induces neuroprotective effects in Aβ-challenged primary cortical neurons

METHODS

Aβ neurotoxicity assay

- Primary rat cortical neurons were treated with the active metabolite of fosgonimeton, fosgo-AM, 100 nM for 15 minutes, challenged with Aβ1-42 15 µM for 24 hours, and immunolabeled for MAP-2 (a neuronal marker) and AT100 (a marker for pTau-Thr212/Ser214). Immunofluorescence analysis was used to determine neuronal survival, neurite network integrity (total neurite length), and pTau levels
- A separate, additional cortical neuron culture was treated with fosgo-AM for 15 minutes and challenged with Aβ1-42 15 µM for 4 hours. Cultures were then immunolabeled with both MAP-2 and MitoSox (a marker of mitochondrial ROS) or both MAP-2 and CytC (a marker of apoptotic signaling)

Cellular signaling assays

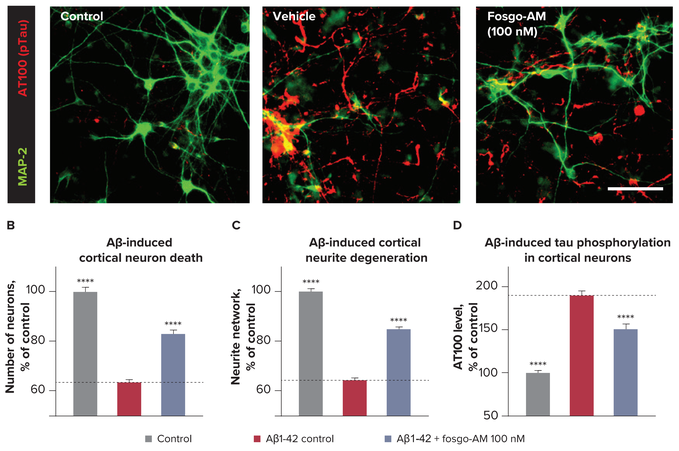
- Primary rat cortical neurons were treated with fosgo-AM 100 nM for 15 minutes and challenged with Aβ1-42 15 µM for 24 hours; western blotting was used to quantify activity of downstream signaling effectors, including total ERK, pERK, total AKT, pAKT (Ser473), total GSK3β, pGSK3β (ratio of pTyr216 to pSer389), and pTau (Thr212/Ser214)
- Western blotting was also used to analyze levels of ULK1 and Beclin-1, key proteins involved in the induction of autophagy, the mechanism by which neurons degrade and clear pathological proteins

Glutamate toxicity assay

- Methods and data were carried out as described in the Supplemental Information (**QR code**)

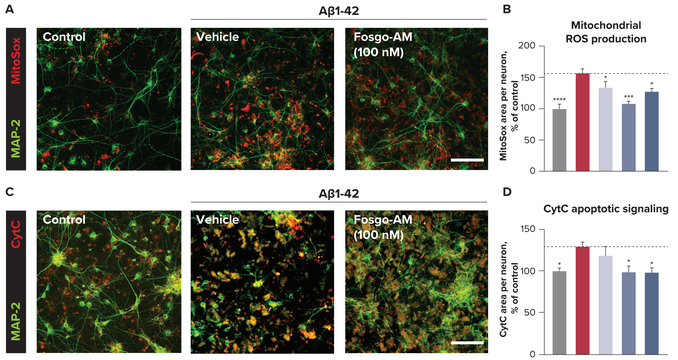
RESULTS

Figure 1. Fosgo-AM attenuates Aβ-induced neuronal death, neurite degeneration, and tau hyperphosphorylation



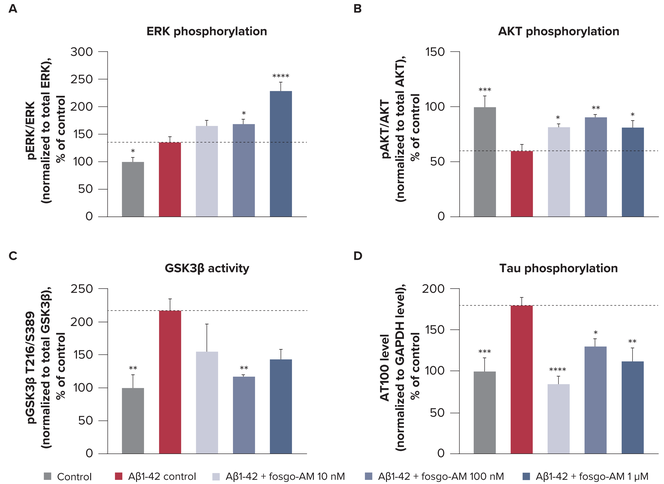
(A) Representative images highlighting the effect of Aβ1-42 on primary cortical neurons in the presence or absence of fosgo-AM; neurons labeled with MAP-2 and AT100 (scale bar = 100 µm). Quantification of (B) neuronal survival (number of MAP-2+ neurons), (C) neurite network (total length of MAP-2+ neurites, in µm), and (D) pTau (overlapping area of AT100 and MAP-2+, in µm) expressed as percentage of healthy controls (100%).
Data presented as mean + SEM; n = 5 or 6. Statistical differences were determined by one-way ANOVA, followed by Fisher's LSD test.
****p < 0.0001 versus Aβ 1-42 control.

Figure 2. Fosgo-AM attenuates Aβ-induced oxidative damage and apoptotic signaling



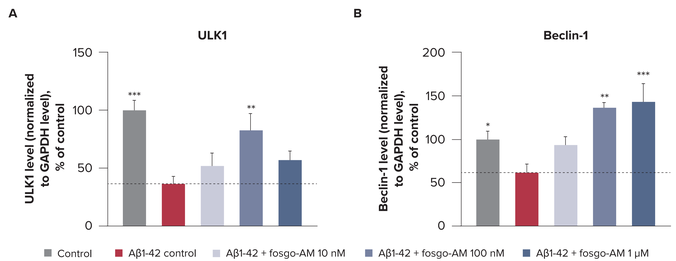
(A) Representative images of primary cortical neurons highlighting the effect of Aβ1-42 on mitochondrial ROS in the presence or absence of fosgo-AM; neurons labeled with MAP-2 and MitoSox (scale bar = 100 µm). (B) Quantification of mitochondrial ROS (MitoSox area per neuron, in µm²) expressed as percentage of healthy controls (100%). (C) Representative images of primary cortical neurons highlighting the effect of Aβ1-42 on CytC release in the presence or absence of fosgo-AM; neurons labeled with MAP-2 and CytC (scale bar = 100 µm). (D) Quantification of CytC release (CytC area per neuron, in µm²) expressed as percentage of healthy controls (100%).
Data presented as mean + SEM; n = 4-6. Statistical differences were determined by one-way ANOVA, followed by Fisher's LSD test.
*p < 0.05, ***p < 0.001, ****p < 0.0001 versus Aβ1-42 control.

Figure 3. Fosgo-AM increases ERK and AKT phosphorylation and reduces GSK3β activity and tau phosphorylation in response to Aβ1-42 injury



Quantification of (A) ERK phosphorylation (pERK/ERK), (B) AKT phosphorylation (pAKT/AKT), (C) GSK3β activity (ratio of active GSK3β to inactive GSK3β), and (D) levels of tau phosphorylation after Aβ1-42 15 µM injury and treatment with fosgo-AM.
Data presented as mean + SEM; n = 4. Statistical differences were determined by one-way ANOVA, followed by Fisher's LSD test.
*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 versus Aβ1-42 control.

Figure 4. Fosgo-AM increases levels of autophagy inducers, ULK1 and Beclin-1



Quantification showing expression levels of (A) ULK1 and (B) Beclin-1 normalized to GAPDH after Aβ1-42 15 µM injury and treatment with fosgo-AM.
Data presented as mean + SEM; n = 4. Statistical differences were determined by one-way ANOVA, followed by Fisher's LSD test.
*p < 0.05, **p < 0.01, ***p < 0.001 versus Aβ1-42 control.

Abbreviations **Aβ**, amyloid beta; **AKT**, protein kinase B; **ANOVA**, analysis of variance; **AD**, Alzheimer's disease; **CytC**, cytochrome C; **ERK**, extracellular signal–regulated kinase; **fosgo-AM**, active metabolite of fosgonimeton; **GAPDH**, glyceraldehyde 3-phosphate dehydrogenase; **GSK3β**, glycogen synthase kinase-3 beta; **HGF**, hepatocyte growth factor; **LSD**, least significant difference; **MAP-2**, microtubule-associated protein 2; **pAKT**, phosphorylated AKT; **pERK**, phosphorylated ERK; **pGSK3β**, phosphorylated glycogen synthase kinase-3 beta; **pTau**, phosphorylated tau; **ROS**, reactive oxygen species; **SEM**, standard error of the mean; **ULK1**, Unc-51–like kinase-1.

References **1.** Alzheimer's Association. *Alzheimers Dement*. 2023;19:1598-1695. **2.** Funakoshi H, Nakamura T. *Curr Signal Transduct Ther*. 2011;6:156-167. **3.** Tyndall SJ, Walikonis RS. *Cell Cycle*. 2006;5:1560-1568. **4.** Johnston JL et al. *Neurotherapeutics*. 2023;20:431-451.

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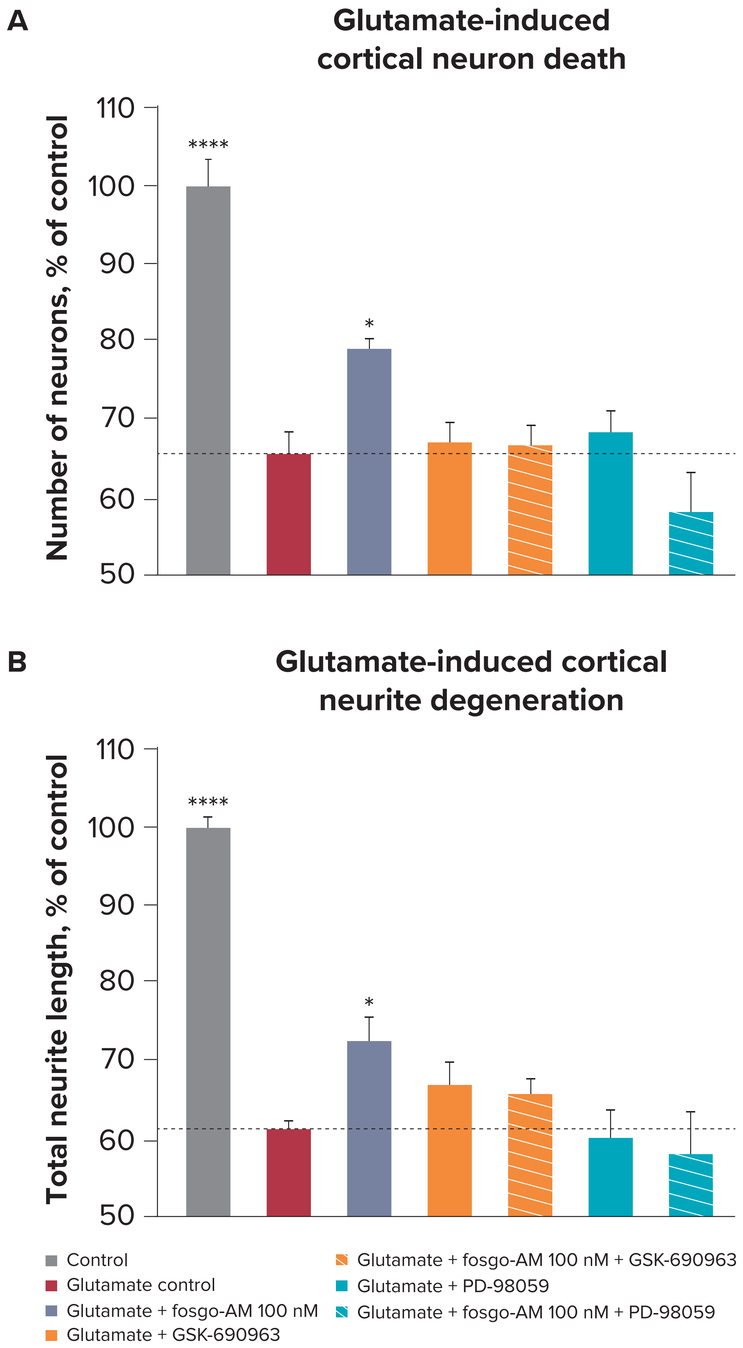
SUPPLEMENTAL INFORMATION

Glutamate toxicity assay

- Primary rat cortical neurons were treated with an AKT inhibitor (GSK-690963) or a MEK/ERK inhibitor (PD-98059), followed by fosgo-AM 100 nM, challenged with glutamate 20 μM for 24 hours, and immunolabeled for MAP-2

Supplemental Figure S1. Neuroprotective actions of fosgo-AM in response to glutamate excitotoxicity are mediated by AKT and MEK/ERK signaling



Quantification of (A) neuronal survival and (B) neurite length after glutamate injury and treatment with fosgo-AM, an AKT inhibitor (GSK-690963), or a MEK/ERK inhibitor (PD-98059).

Data presented as mean + SEM; n = 4-6. Statistical differences were determined by one-way ANOVA, followed by Dunnett's test.

*$p < 0.01$, ****$p < 0.0001$ versus glutamate control.

Abbreviations **AKT**, protein kinase B; **ANOVA**, analysis of variance; **ERK**, extracellular signal–regulated kinase; **fosgo-AM**, active metabolite of fosgonimeton; **GSK**, glycogen synthase kinase; **MAP-2**, microtubule-associated protein 2; **MEK**, mitogen-activated protein kinase; **SEM**, standard error of the mean.

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