Neuroprotective mechanisms of fosgonimeton against excitotoxicity in primary neuron culture

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CONCLUSIONS

1. **Fosgo-AM enhances PI3K/AKT signaling following glutamate injury in cortical neurons**
2. **Fosgo-AM promotes the inactivation of pTau-inducing GSK3β and increases expression of mitoprotective Bcl-2 protein**
3. **Neuroprotective effects of fosgo-AM are mediated by pro-survival AKT/S6K signaling**

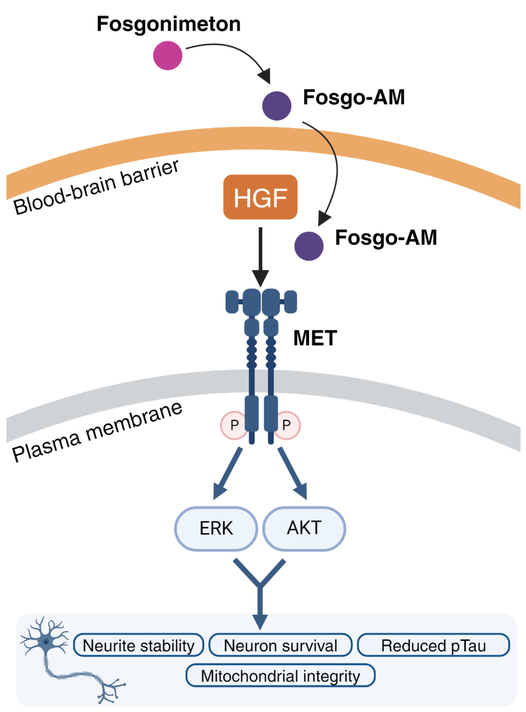
KEY TAKEAWAY

The neuroprotective effects of fosgonimeton against glutamate excitotoxicity in vitro are driven, in part, by activation of pro-survival signaling pathways that may help to counteract neurodegenerative hallmarks such as mitochondrial dysfunction and tau pathology

INTRODUCTION

- AD is a progressive neurodegenerative disease characterized by the abnormal accumulation of pathological proteins such as Aβ and pTau in the brain, and the presence of oxidative stress, glutamate excitotoxicity, and neuroinflammation[1,2]
- Glutamate excitotoxicity produces AD-related pathological effects in neurons, including intracellular Ca^{2+} toxicity, accumulation of toxic pTau, mitochondrial dysfunction, oxidative stress, and apoptosis[3]
- The neurotrophic HGF system exhibits multimodal neuroprotective effects that can counteract excitotoxic mechanisms via activation of pro-survival signaling mediated by PI3K/AKT[4]
- Fosgonimeton is a small-molecule positive modulator of the neurotrophic HGF system that has demonstrated neuroprotective and anti-inflammatory effects in preclinical models of dementia[5]
- Fosgonimeton is under clinical investigation for safety and efficacy in the treatment of mild to moderate AD (NCT04488419).

Fosgonimeton positively modulates the neurotrophic HGF system



Fosgonimeton is converted to the active metabolite (Fosgo-AM) following administration

OBJECTIVE

To characterize the neuroprotective mechanism of action of fosgonimeton against glutamate excitotoxicity

METHODS

Phospho-MET assay

- Rat primary cortical neurons were treated with HGF (0.05 ng/ml) or fosgo-AM + HGF (0.05 ng/ml) for 15 minutes, and western blot was used to quantify levels of total MET and pMET (Y1234/Y1235).

Cell signaling assays

- Rat primary cortical neurons were treated with fosgo-AM (100 nM) for 15 minutes, challenged with glutamate (20 µM), and western blot was used to quantify levels of total PI3K, pPI3K, total AKT, pAKT (Thr308), total Bcl-2, total GSK3β, pGSK3β (Ser9), and GAPDH at 1, 6, and 24 hours post glutamate challenge. All conditions included HGF (0.05 ng/ml).

Phospho-tau and MitoROS assays

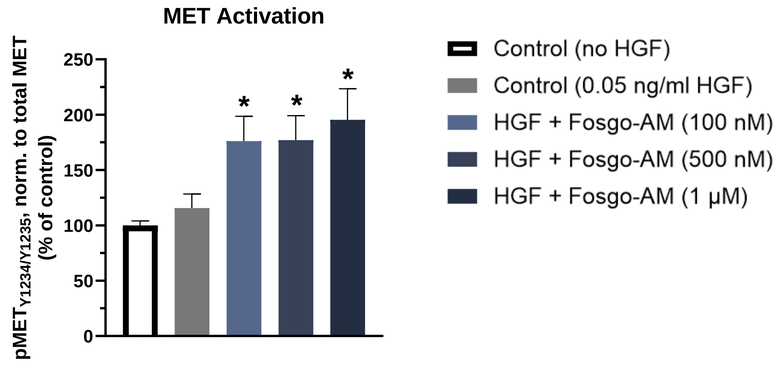
- Rat primary cortical neurons were treated with fosgo-AM (100 nM) for 15 minutes, challenged with glutamate (20 µM) for 24 hours, immunolabeled for MAP-2 and either AT100 (a marker for pTau-Thr212/Ser214) or MitoSox (a marker of mitochondrial ROS). Automated image analysis was used to determine pTau levels per neuron or ROS produced by mitochondria. All conditions included HGF (0.05 ng/ml).

Survival and network assay – AKT/S6K inhibition

- Rat primary cortical neurons were treated with GSK-690963 (100 nM; AKT inhibitor) for 40 minutes, followed by fosgo-AM (100 nM) treatment for 20 minutes.
- In another culture, primary cortical neurons were treated with PF-4708671 (5 µM; S6K inhibitor) for 45 minutes, followed by fosgo-AM (100 nM) treatment for 15 minutes.
- Cultures were then challenged with glutamate (20 µM) for 24 hours, immunolabeled for MAP-2 (a neuronal marker), and automated analysis was used to determine neuronal survival or neurite network integrity. All conditions included HGF (0.05 ng/ml).

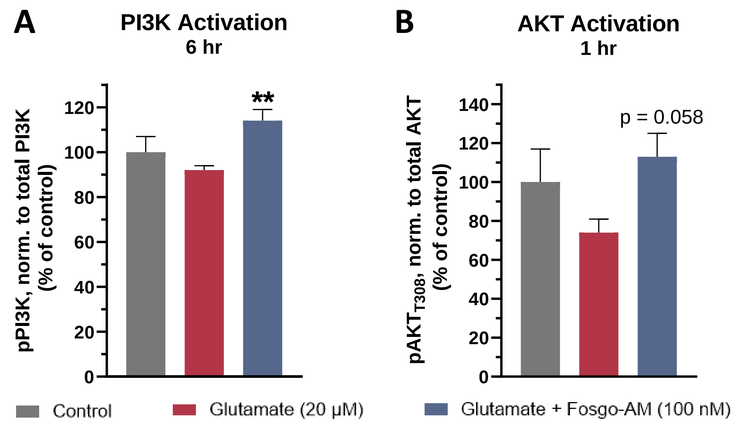
RESULTS

Figure 1. Fosgo-AM promotes HGF-mediated activation of the MET receptor in primary cortical neurons



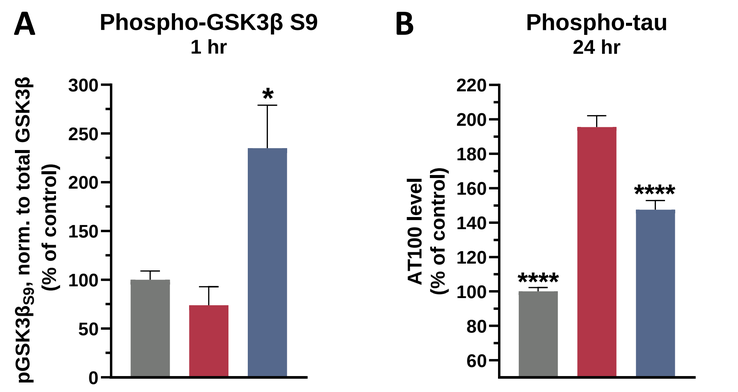
Quantification of MET activation 15 minutes post treatment expressed as percentage of control (100%). Data presented as mean + SEM; n = 3-4. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus HGF control. *p <0.05

Figure 2. Fosgo-AM enhances PI3K/AKT signaling following glutamate injury



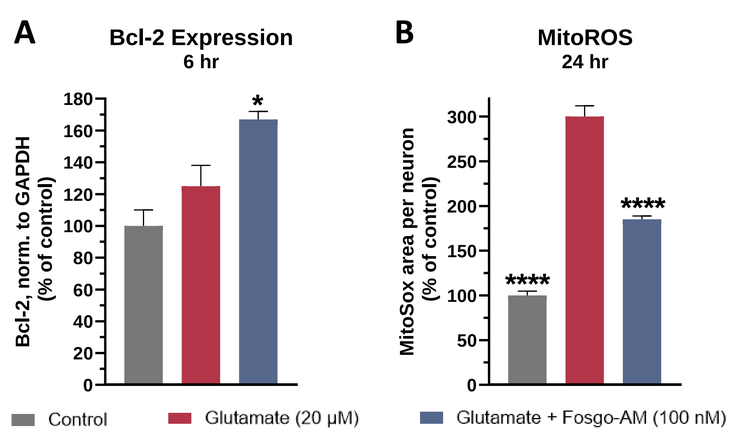
Quantification of (A) PI3K activation 6h post glutamate and (B) AKT activation 1h post glutamate expressed as percentage of normal control (100%). Data presented as mean + SEM; n = 3-4. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus glutamate control. **p <0.01

Figure 3. Fosgo-AM promotes phospho-inactivation of GSK3β and reduces pTau following glutamate injury



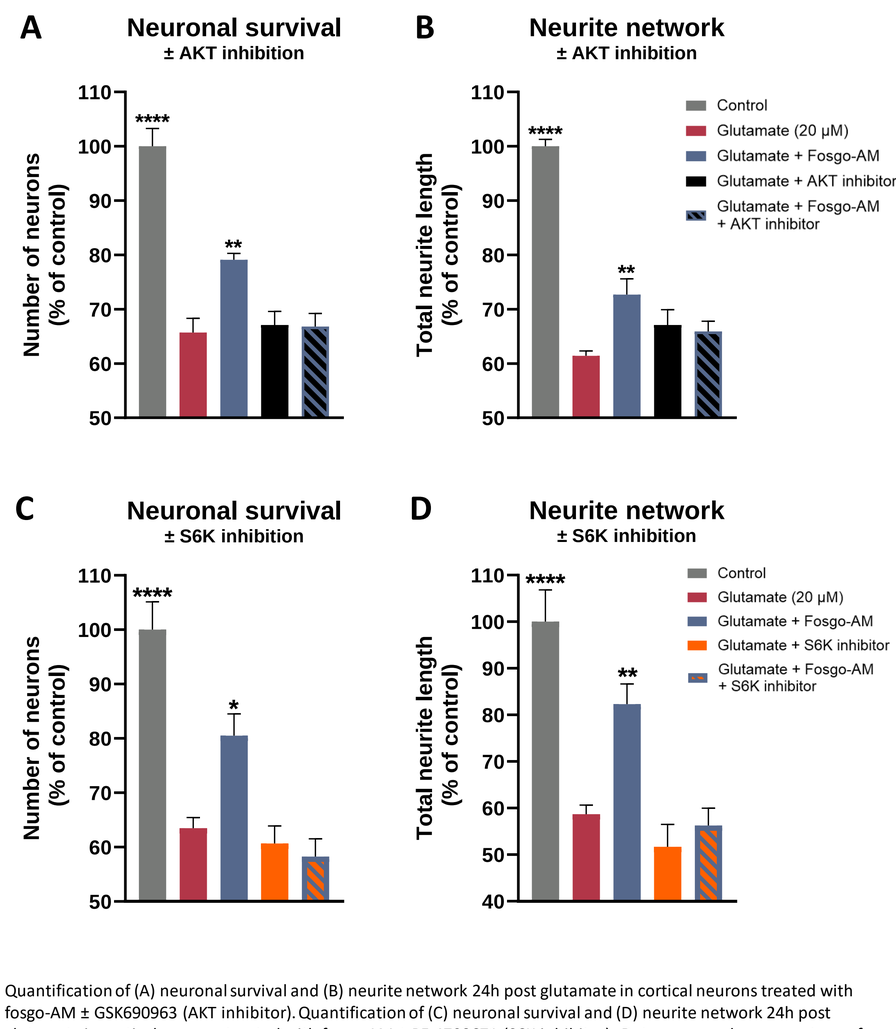
Quantification of (A) Phospho-GSK3β (Ser9) 1h post glutamate and (B) Phospho-tau 24h post glutamate expressed as percentage of normal control (100%). Data presented as mean + SEM; n = 3-4 for GSK3β and n = 5-6 for Phospho-tau. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus glutamate control. *p <0.05, ****p <0.0001

Figure 4. Fosgo-AM upregulates Bcl-2 expression and attenuates mitochondrial ROS following glutamate injury



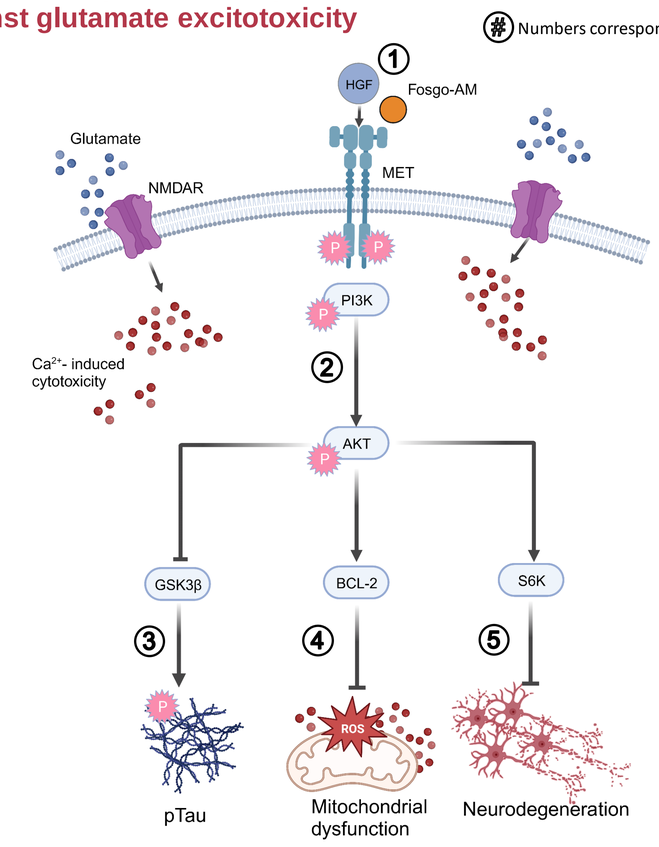
Quantification of (A) Bcl-2 expression 6h post glutamate and (B) mitochondrial ROS 24h post glutamate expressed as percentage of normal control (100%). Data presented as mean + SEM; n = 3-4 for Bcl-2; n = 5-6 for MitoROS. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus glutamate control. *p <0.05, ****p <0.0001

Figure 5. Neuroprotective effects of fosgo-AM involve activation of pro-survival signaling mediated by AKT/S6K axis



Quantification of (A) neuronal survival and (B) neurite network 24h post glutamate in cortical neurons treated with fosgo-AM ± GSK690963 (AKT inhibitor). Quantification of (C) neuronal survival and (D) neurite network 24h post glutamate in cortical neurons treated with fosgo-AM ± PF-4708671 (S6K inhibitor). Data expressed as percentage of normal control (100%) and presented as mean + SEM; n = 5-6. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus glutamate control. *p <0.05, **p <0.01, ****p <0.0001

Figure 6. Proposed neuroprotective mechanisms of fosgonimeton against glutamate excitotoxicity



Abbreviations: **Aβ**, amyloid beta; **AD**, Alzheimer's disease; **AKT**, protein kinase B; **ANOVA**, analysis of variance; **Bcl-2**, B-cell lymphoma; **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; **p**, phosphorylation; **pAKT**, phosphorylated AKT; **PI3K**, phosphoinositide 3-kinase; **pGSK3β**, phosphorylated glycogen synthase kinase-3 beta; **pPI3K**, phosphorylated PI3K; **pTau**, phosphorylated tau; **ROS**, reactive oxygen species; **S6K**, ribosomal S6 kinase; **SEM**, standard error of the mean.

References: **1**. Alzheimer's Association. *Alzheimers Dement*. 2023;19(4):1598-1695. **2.** Dar KB et al. *Cell Mol Neurobiol*. 2020;40(3):313-345. **3**. Wang R et al. *J Alzheimers Dis*. 2017;57(4):1041-1048. **4**. Xiao G-H et al. *Proc Natl Acad Sci*. 2001;98(1):247–52. **5.** Johnston JL et al. *Neurotherapeutics*. 2023;20(2):431-451.

Acknowledgments

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Disclosures

Sherif M. Reda, Wei Wu, Andrée-Anne Berthiaume, Sharay E. Setti, Robert W. Taylor, Jewel L. Johnston, and Kevin J. Church are employees and stockholders 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.