Poster #49

Fosgonimeton attenuates amyloid-β-induced autophagic impairment in primary cortical neurons

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CONCLUSIONS

1. Following Aβ1-42 injury in primary cortical neurons, fosgo-AM enhances neuronal survival and reduces pTau, and such effects are mediated by positively modulating the neurotrophic HGF system
2. Fosgo-AM increases levels of autophagy inducers, ULK1 and Beclin-1, following Aβ1-42 injury
3. Fosgo-AM reduces abnormal accumulation of autophagic markers (i.e., autophagic stress) following Aβ1-42 injury

KEY TAKEAWAY

The data presented demonstrate the ability of fosgonimeton to potentially help address autophagic impairment in AD, which may lead to the reduction of pTau pathology with treatment

Abbreviations: **Aβ**, amyloid beta; **AD**, Alzheimer's disease; **AKT**, protein kinase B; **ANOVA**, analysis of variance; **ERK**, Extracellular signal regulated kinase; **fosgo-AM**, active metabolite of fosgonimeton; **GAPDH**, glyceraldehyde 3-phosphate dehydrogenase; **HGF**, hepatocyte growth factor; **LAMP2**, lysosomal membrane-associated protein 2; **LC3**, Microtubule-associated proteins 1A/1B light chain 3B; **LSD**, least significant difference; **MAP2**, microtubule-associated protein 2; **p**, phosphorylation; **pTau**, phosphorylated tau; **SEM**, standard error of the mean; **SEM**, Unc-like kinase 1, **ULK1**

References: **1**. Stavoe AKH et al. *Annu Rev Cell Dev Biol*. 2019;35(1):477-500. **2**. Uddin MS et al. *Front Aging Neurosci*. 2018;10(4). **3**. Piras A et al. *Acta Neuropathologica Communications* (2016);4:22. **4**. Pickford F et al. *J Clin Ivest*. 2008;118(6):2190-2199. **5.** Reda SM et al. *Neurotherapeutics*. 2024:21(4):e00350.

Acknowledgments

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Disclosures

Sherif M. Reda, Wei Wu, Andrée-Anne Berthiaume, Sharay E. Setti, Jewel L. Johnston, Robert W. Taylor, 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.

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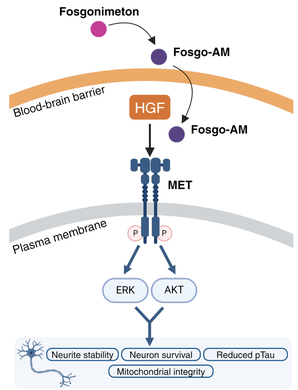
INTRODUCTION

- Autophagy is a highly dynamic, lysosome-based degradation process that promotes the clearance of degenerative factors (e.g., pTau) to maintain cellular functions, preserve metabolic integrity, and ensure survival [1]
- In AD, impaired neuronal autophagy can lead to the abnormal accumulation of autophagic vesicles (e.g., autophagosomes, lysosomes), failure to degrade pTau and build-up of toxic aggregates, dysregulated homeostasis, synaptic dysfunction, and neurotoxicity [2]
  - In post-mortem AD brains, there is an abnormal accumulation of autophagosome marker LC3 and lysosomal marker LAMP2, as well as increased co-localization of pTau and autophagosomes [3]
  - Levels of Beclin-1, a key protein involved in the induction of autophagy, are reduced in the brains of people with AD [4]
- We have previously demonstrated that fosgonimeton, a small-molecule positive modulator of the neurotrophic hepatocyte growth factor (HGF) system, is neuroprotective and reduces pTau levels in Aβ-driven preclinical models of AD [5]
- Herein, we investigate the effect of fosgonimeton on autophagic function in cortical neurons following Aβ injury to gain a better understanding of the neuroprotective effects of fosgonimeton treatment, specifically in the context of reducing pTau
- 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 systemic administration. Fosgo-AM positively modulates HGF activity and promotes MET (HGF receptor) activation

OBJECTIVE

To investigate the impact of fosgonimeton on Aβ-induced autophagic impairment in primary cortical neurons

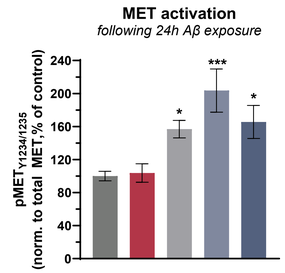
METHODS

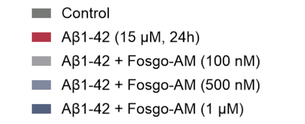
Aβ1-42 neurotoxicity assay

- Rat primary cortical neurons were harvested from E14 rat embryos and cultured for 12 days
- Cultures were treated with vehicle (containing HGF 0.05 ng/ml) or fosgo-AM for 15 minutes, and then challenged with Aβ1-42 15 µM for 24 hours
- After 24 hours, immunostaining was used to assess the following metrics:
  - Neuronal survival (MAP2+ positive neurons),
  - Neurite network integrity (total length of MAP2+ neurites, in µm)
  - pTau (Ser202/Thr205) levels (overlap area of AT8 and MAP2+ neurons)
  - LC3+ autophagosomes (overlap area of LC3 and MAP2+ neurons)
  - pTau-autophagosome co-localization (overlap area of pTau-LC3)
  - LAMP2+ lysosomes (overlap area of LAMP2 and MAP2+ neurons)
  - Autophagosome-lysosome co-localization (overlap area of LC3-LAMP2)
- Western blot analysis was used to evaluate levels of pMET (Tyr1234/1235), total MET, ULK1, Beclin-1, and GAPDH (reference protein)

RESULTS

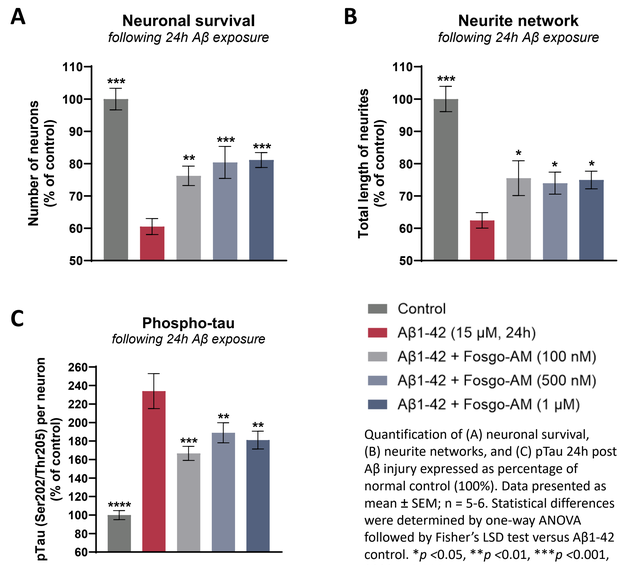
Figure 1. Fosgo-AM promotes activation of the MET receptor in primary cortical neurons following Aβ1-42 injury





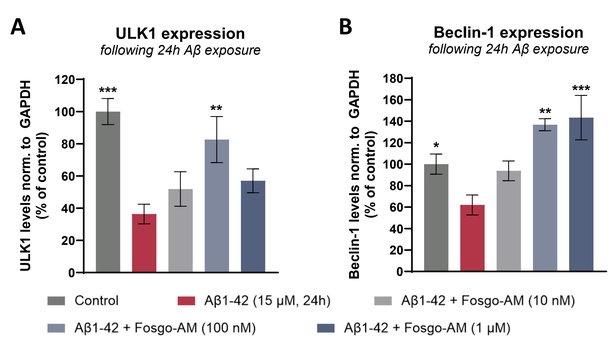
Quantification of MET phosphorylation 24h post Aβ1-42 treatment expressed as percentage of control (100%). Data presented as mean ± SEM; n = 3-4. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus Aβ1-42 . *p <0.05, *** p <0.001

Figure 2. Fosgo-AM promotes neuronal survival, preserves neurite networks, and reduces pTau following Aβ1-42 injury



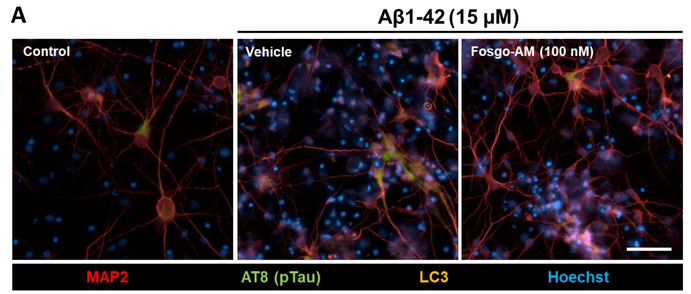
Quantification of (A) neuronal survival, (B) neurite networks, and (C) pTau 24h post Aβ injury expressed as percentage of normal control (100%). Data presented as mean ± SEM; n = 5-6. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus Aβ1-42 control. *p <0.05, **p <0.01, ***p <0.001, ****p <0.0001

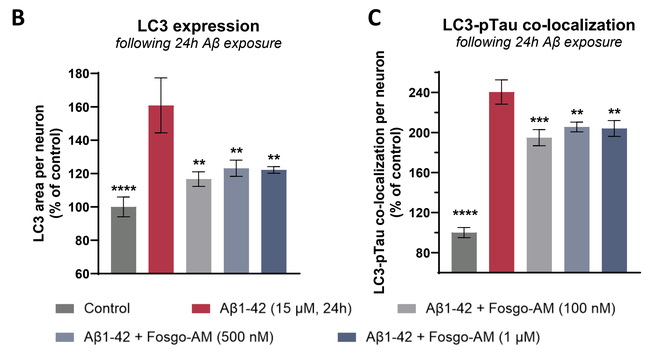
Figure 3. Fosgo-AM increases levels of autophagy inducers, ULK1 and Beclin-1, following Aβ1-42 injury



Quantification of (A) ULK1 expression and (B) Beclin-1 expression 24h post Aβ injury normalized to GAPDH and 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 Aβ1-42 control. *p <0.05, **p <0.01, ***p <0.001

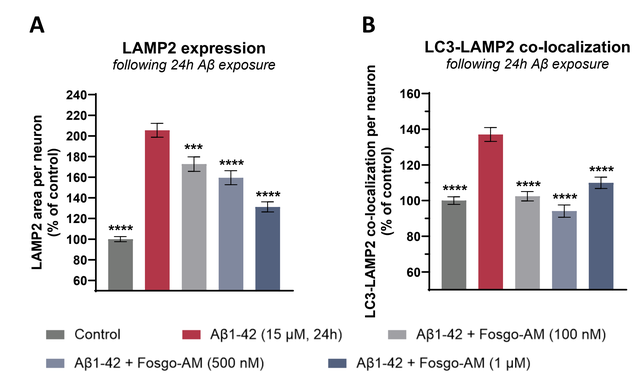
Figure 4. Fosgo-AM reduces abnormal accumulation of autophagosomes and co-localization of pTau with autophagosomes following Aβ1-42 injury





(A) Representative images of primary cortical neurons highlighting the effect of Aβ1-42 on MAP2 (neuronal marker), AT8 (marker for pTau) and LC3 (marker for autophagosomes) in the presence or absence of fosgo-AM. Scale bar = 50 µm. Quantification of (B) LC3+ autophagosomes and (C) LC3-pTau co-localization 24h post Aβ injury expressed as percentage of normal control (100%). Data presented as mean ± SEM; n = 5-6. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus Aβ1-42 control. **p <0.01, ***p <0.001, ****p <0.0001

Figure 5. Fosgo-AM reduces abnormal accumulation of lysosomes and co-localization of autophagosomes with lysosomes following Aβ1-42 injury



Quantification of (A) LAMP2+ lysosomes and (B) LC3-LAMP2 co-localization 24h post Aβ injury expressed as percentage of normal control (100%). Data presented as mean ± SEM; n = 5-6. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus Aβ1-42 control. ***p <0.001, ****p <0.0001