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 increased the number of neurons in culture and enhanced neurite outgrowth

2 Cortical neurons treated with fosgo-AM exhibited significant protection 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, the active metabolite of fosgonimeton, highlight its therapeutic potential to restore neuronal health and slow or reverse neurodegeneration

INTRODUCTION

Figure 1. Positive modulators of the HGF/MET pathway may counteract neurodegeneration through multiple mechanisms

- HGF signaling through the MET receptor promotes neuronal growth and survival from prenatal development through adulthood[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 (ACT-AD, NCT04491006; 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 assess the neuroprotective effects of fosgo-AM in vitro 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 (1.0 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
  - Primary neuron cultures contain small amounts of HGF,[4] the activity of which fosgo-AM modulates
- The Cell Titer-Glo luminescent cell viability assay (Promega), which measures ATP levels, was used to calculate cell viability for each experimental group
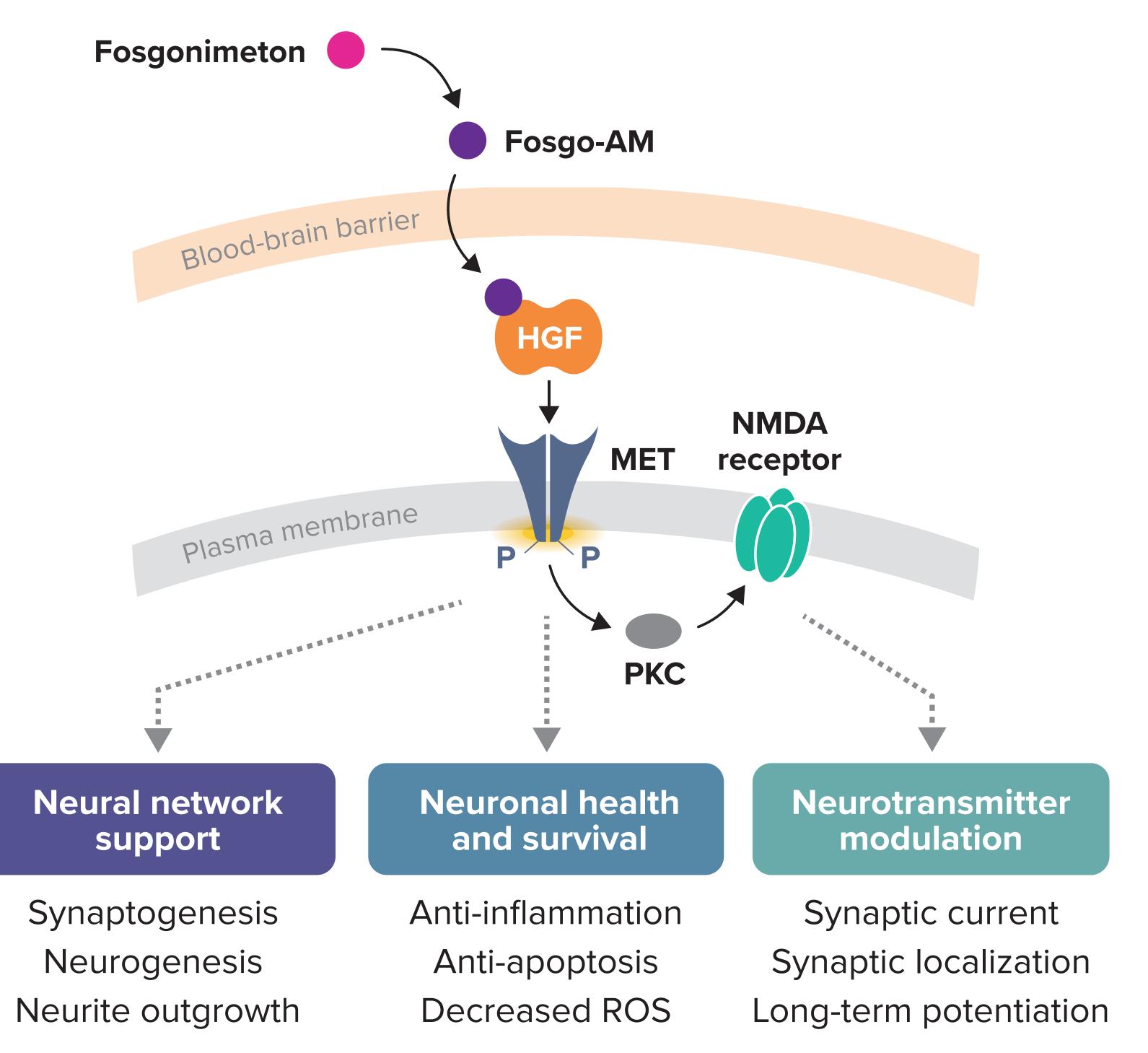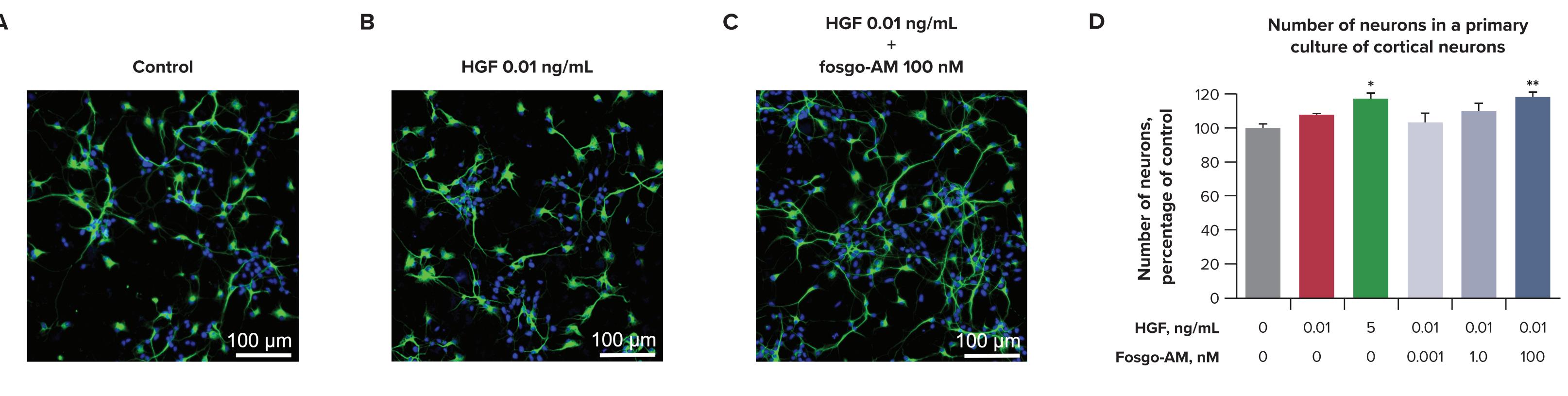
MET occlusion assay
- Primary cortical neurons from rat embryos at embryonic day 15 were cultured for 12 days before testing
- Day 13 neuronal cultures were preincubated for 60 minutes with one of the following inhibitors of MET-associated molecules (or vehicle): GSK-690963 (100 nM; AKT inhibitor), calphostin C (200 nM; PKC inhibitor), KN-62 (2 µM; CamKII inhibitor), or PD98059 (10 µM; MEK inhibitor) (**supplemental materials, QR code**)
- 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 the Tukey 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.
Statistical significance was determined by 1-way ANOVA with the Dunnett posttest.
*P < 0.05; **P < 0.01 compared with control (grey).

- Under basal conditions, the number of MAP2-positive neurons and total length of neurite network present after 48-hours incubation was significantly increased with 5 ng/mL HGF as well as 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 (**supplemental materials, QR code**)

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 the Tukey posttest.
*P < 0.05; **P < 0.01; ***P < 0.001 compared with insult (red).

- Treatment with fosgo-AM at all evaluated/tested 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**) GSK-690963, (**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 the Tukey posttest.
*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 compared with insult (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 GSK-690963 (AKT inhibition), calphostin C (PKC inhibition), and PD98059 (MEK inhibition) (**supplemental materials, QR code**)
- 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; **fosgo-AM**, active metabolite of fosgonimeton; H_2O_2, hydrogen peroxide; **HGF**, hepatocyte growth factor; **HTRF**, homogeneous time-resolved fluorescence; **LPS**, lipopolysaccharide; **MAP2**, microtubule-associated protein 2; **MEK**, mitogen-activated protein kinase kinase; MPP^+, 1-methyl-4-phenylpyridinium; **NMDA**, N-methyl-D-aspartate; **P**, phosphorylation; **PKC**, protein kinase C; **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.** Gutierr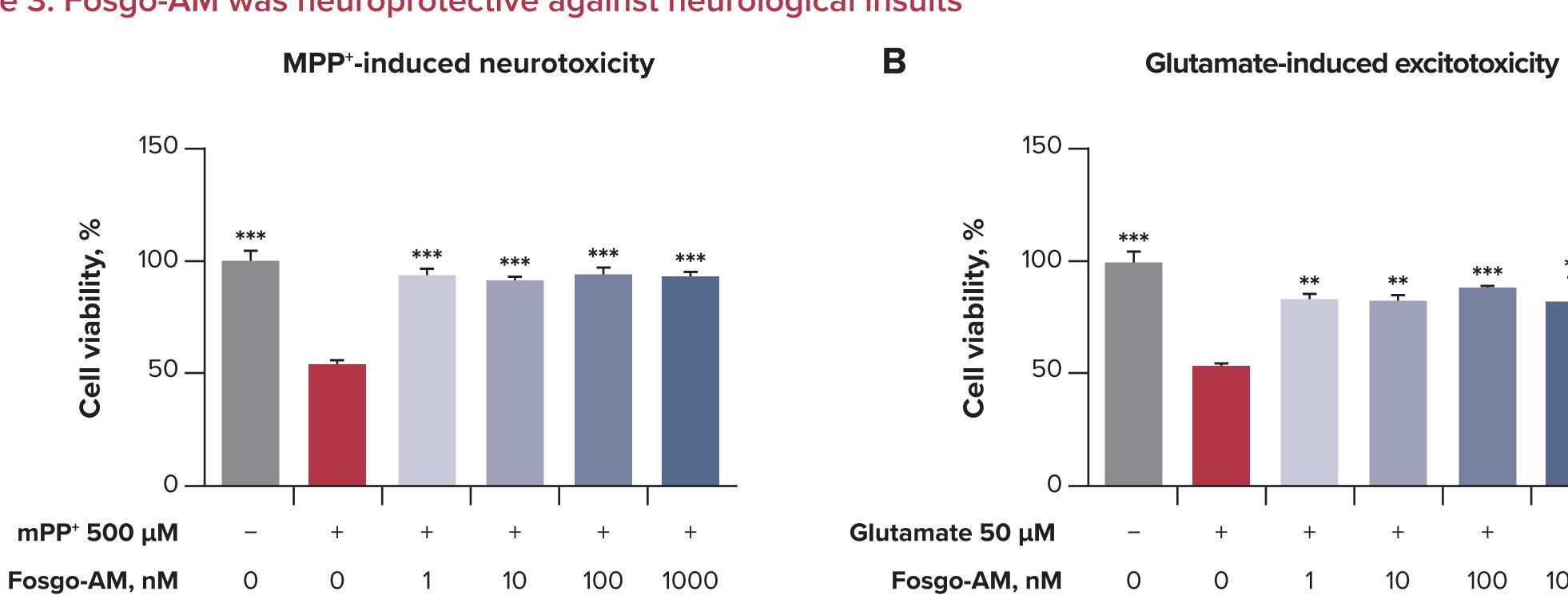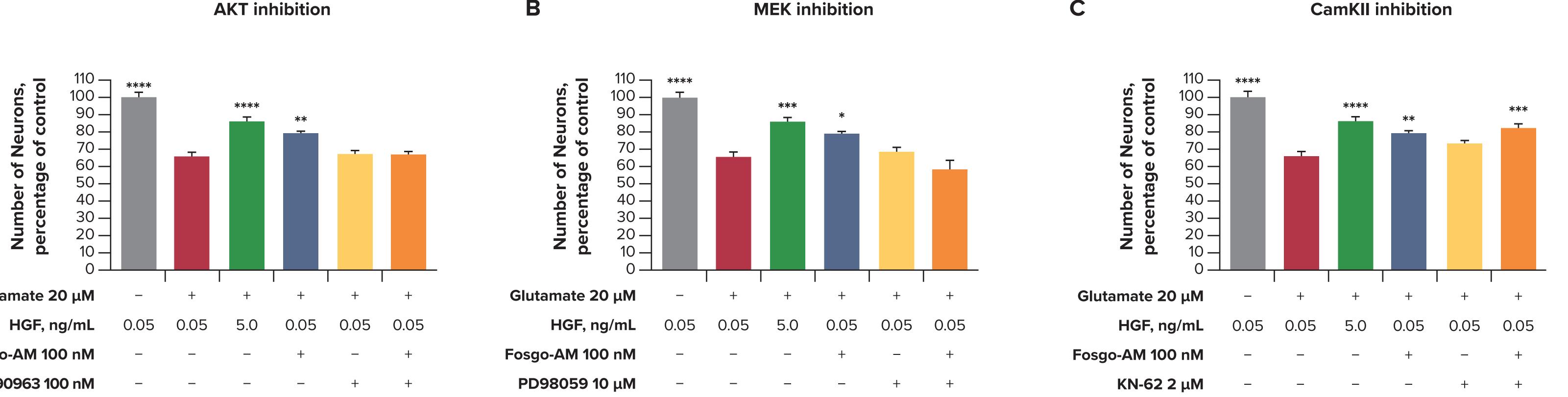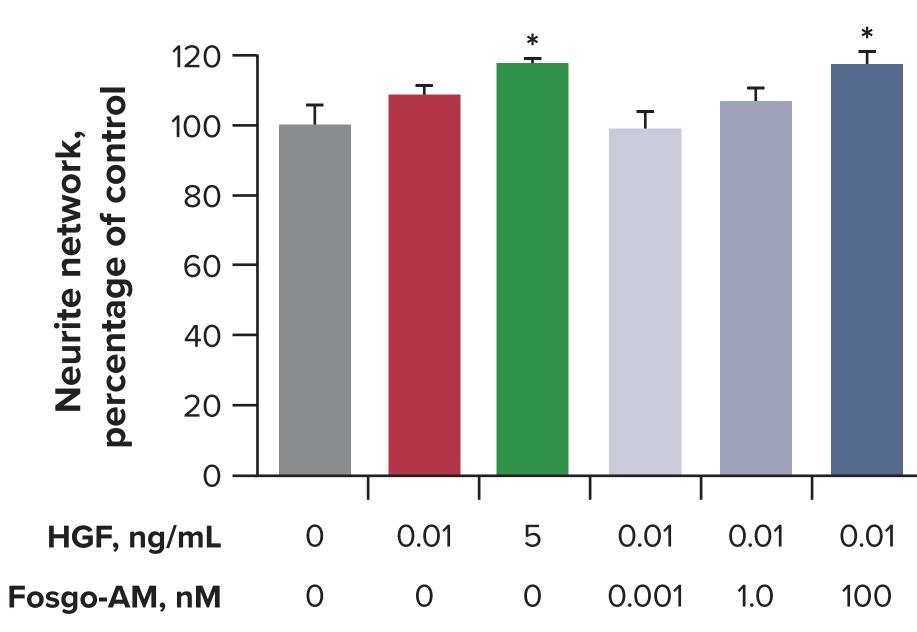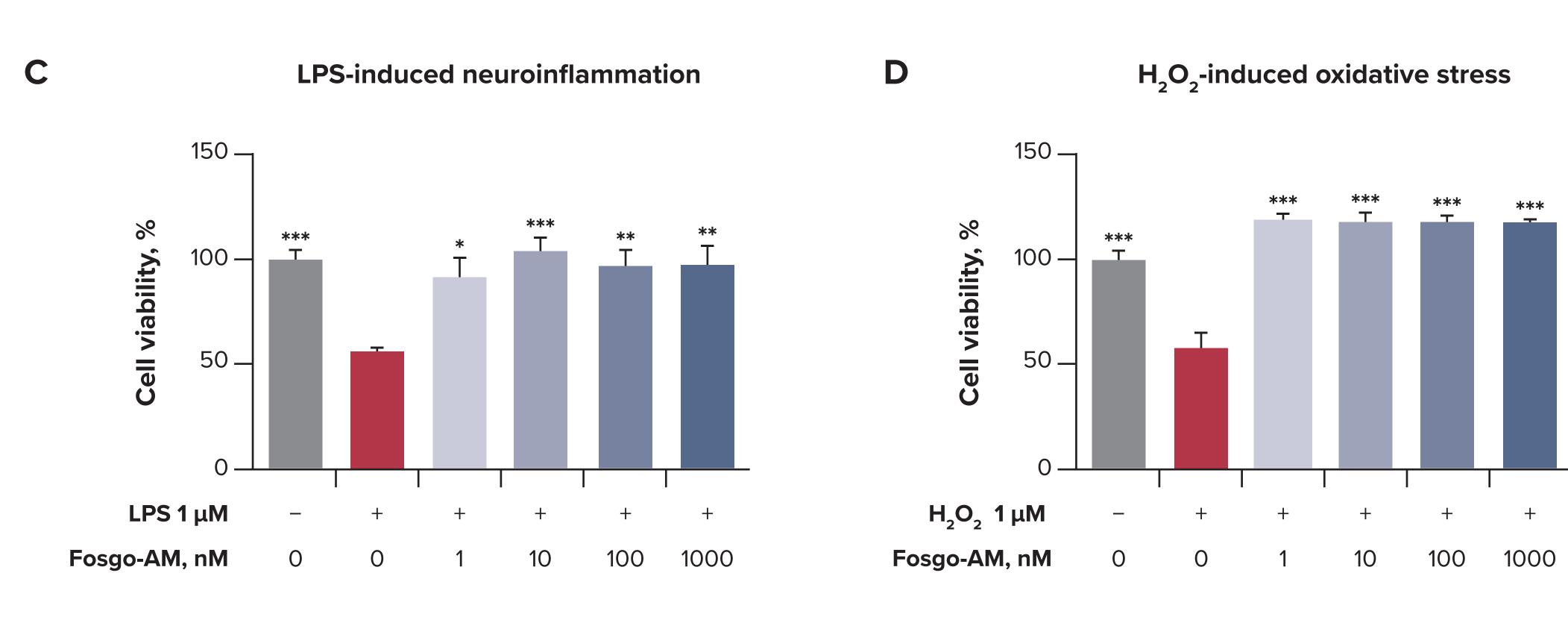ez H et al. Development. 2004;131:3717-3726.