ATH-1020, a novel small-molecule positive modulator of the neurotrophic HGF system, is neuroprotective and improves motor function in preclinical models of Parkinson's disease

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CONCLUSIONS

1. **ATH-1020 is a small-molecule positive modulator of the neurotrophic HGF signaling system**
2. **ATH-1020 induces neurotrophic effects (neurite outgrowth and synaptogenesis) that may indicate repair potential in PD**
3. **ATH-1020 is neuroprotective against a variety of neurotoxic insults relevant to PD and has anti-inflammatory effects**
4. **Treatment with ATH-1020 improves motor function in a 6-OHDA rat model of PD**

KEY TAKEAWAY

These data highlight the effects of ATH-1020 against PD-related pathology, supporting positive modulation of the neurotrophic HGF system as a potential treatment approach for PD.

Disclosures

Wei Wu, Sherif M. Reda, 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

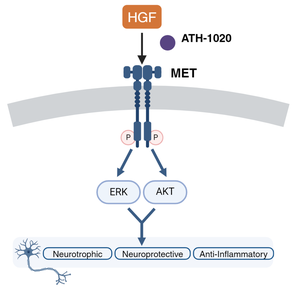
ATH-1020 is an investigational therapy and has not received FDA approval nor been demonstrated to be safe or effective for any use.

Presented at SfN; October 5 -9, 2024; Chicago, IL

INTRODUCTION

- PD is a neurodegenerative disorder that involves oxidative stress, mitochondrial dysfunction, neuroinflammation, and degeneration of dopaminergic neurons, ultimately resulting in motor dysfunction[1]
- The neurotrophic HGF system represents a promising therapeutic target for neurodegenerative diseases including PD due to its multimodal, neurotrophic, neuroprotective, and anti-inflammatory effects[2-4]
- We have developed a series of small-molecule positive modulators of the neurotrophic HGF system to explore the potential of this therapeutic strategy.
- Here, we investigate the effect of the brain penetrant, orally bioavailable HGF positive modulator, ATH-1020, on key components of PD pathology including neurodegeneration, inflammation, and motor deficits in preclinical models.

Figure 1. ATH-1020 positively modulates the neurotrophic HGF system



OBJECTIVE

To investigate the therapeutic potential of ATH-1020 as an approach for neurogenerative diseases, including PD, in preclinical models

METHODS

MET and ERK activation assay

- HEK293 cells were treated with ATH-1020 in conjunction with low doses of HGF (1 ng/ml or 0.1 ng/ml) in the presence or absence of a MET receptor inhibitor, capmatinib, for 15 minutes.
- Cell lysates were collected and evaluated for phosphorylated-MET (Y1234/1235) via pMET sandwich ELISA kit and phosphorylated ERK (T202/Y204) via pERK HTRF kit.

Neurotrophic assay

- Primary rat hippocampal neurons were treated with ATH-1020 every other day for 3 or 9 days.
- Neurite outgrowth was assessed on day 3 via immunostaining with beta-III tubulin antibody. Synaptogenesis was assessed on day 9 via immunostaining with synaptobrevin-II antibody.

Neuroprotection assay

- Primary rat cortical neurons were pretreated with vehicle (containing 5 ng/ml HGF) or ATH-1020 (1, 10, 100 or 1000 nM) for 15 minutes, then challenged with one of the following neurotoxic compounds: H_2O_2, glutamate, LPS or MPP^+ for 24 hours.
- The Cell Titer-Glo luminescent cell viability assay (Promega) was used to calculate cell viability for each experimental group.

LPS-challenged THP-1 macrophages assay

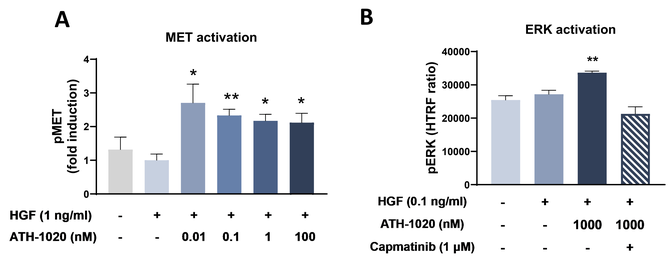
- THP-1 differentiated macrophages were pretreated for 20 minutes with ATH-1020 (1, 10, 100, or 1000 nM) and then challenged with 50ng/ml LPS for 24 hours.
- Proinflammatory cytokines IL-1β, IL-6 and TNF-α were measured via HTRF.

6-OHDA rat model of PD

- 8-to-9-week-old male Sprague-Dawley rats received unilateral striatal injections of either 6-OHDA or saline vehicle. After approximately 2 weeks of recovery from 6-OHDA lesion or sham surgery, ATH-1020 (0.5, 2, or 8 mg/kg) was orally administered daily for 6 weeks (study days 15 to 56).
- 8 weeks post-surgery (day 56), motor function was assessed using accelerating rotarod (4 – 400 rpm in 300 sec) and grip strength tests. For each test, 3 trials were averaged per animal.

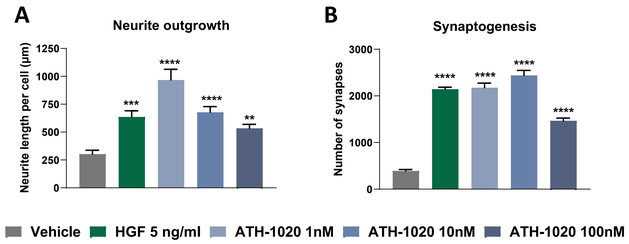
RESULTS

Figure 2. ATH-1020 enhances HGF-mediated MET phosphorylation and activates the downstream ERK signaling cascade



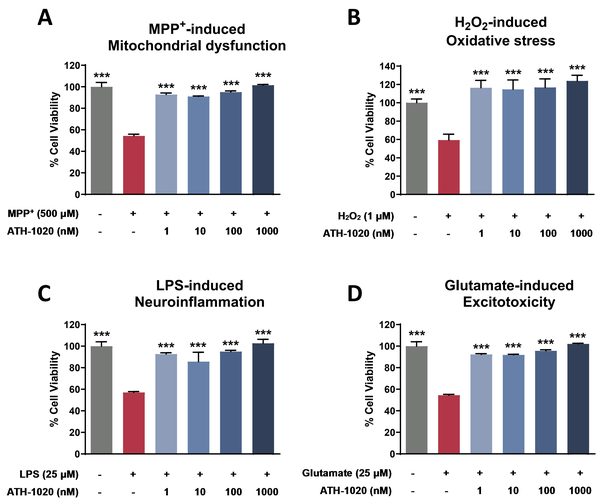
Levels of **(A)** MET (Y1234/1235) phosphorylation and **(B)** ERK (T202/Y204) phosphorylation in HEK293 cells after treatment with ATH-1020 at different concentrations in the presence or absence of capmatinib (MET inhibitor). Data presented as mean + SEM; n=3-6 per group. Statistical significance was determined by one-way ANOVA with Tukey post-test. *p<0.05, **p<0.01, ***p<0.001 vs HGF alone.

Figure 3. ATH-1020 treatment has neurotrophic effects promoting neurite outgrowth and synaptogenesis in vitro



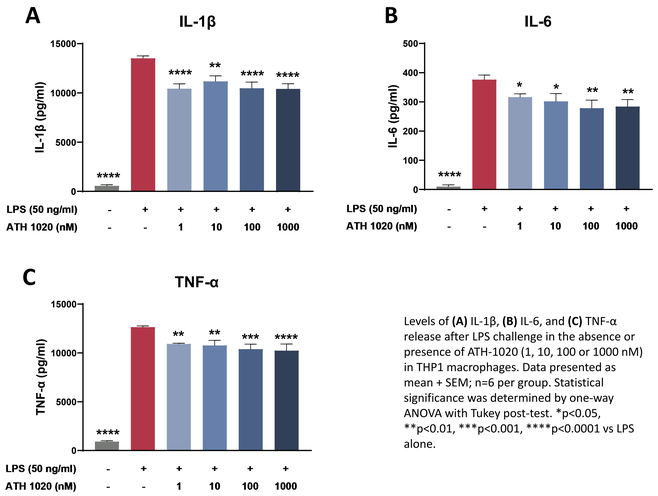
(A) Neurite length per cell and **(B)** synaptic count following treatment with vehicle, HGF, or ATH-1020 (1, 10 or 100 nM) in primary hippocampal neurons. Data presented as mean + SEM. 10 images were analyzed per well for each treatment condition; n=3 wells per group. Statistical significance was determined by one-way ANOVA with Tukey post-test. **p<0.01, ***p<0.001, ****p<0.0001 vs vehicle alone.

Figure 4. ATH-1020 treatment is neuroprotective against a variety of neurotoxic insults in vitro



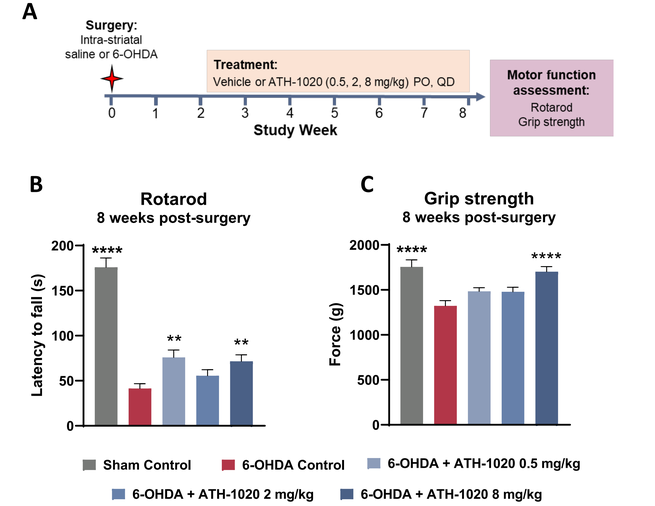
Percentage of cortical neurons surviving in culture after exposure to the following neurotoxic insults: **(A)** MPP^+, **(B)** H_2O_2, **(C)** LPS, and **(D)** Glutamate, in the presence or absence of ATH-1020 (1, 10, 100, or 1000 nM). Data presented as mean + SEM. Statistical significance was determined by one-way ANOVA with Tukey post-test. *p<0.05, **p<0.01, ***p<0.001 vs insult alone.

Figure 5. ATH-1020 reduces pro-inflammatory cytokine secretion in LPS-challenged THP1 macrophages



Levels of **(A)** IL-1β, **(B)** IL-6, and **(C)** TNF-α release after LPS challenge in the absence or presence of ATH-1020 (1, 10, 100 or 1000 nM) in THP1 macrophages. Data presented as mean + SEM; n=6 per group. Statistical significance was determined by one-way ANOVA with Tukey post-test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs LPS alone.

Figure 6. Treatment with ATH-1020 improves motor function in a 6-OHDA rat model of PD



(A) Schematic representation of study design for testing ATH-1020 treatment in the 6-OHDA rat model of PD. Quantification of motor function via **(B)** rotarod measured as latency to fall, in seconds, and **(C)** forelimb grip strength, in grams of force, following 6 weeks of daily oral treatment with vehicle or ATH-1020 (0.5, 2, or 8 mg/kg). Data presented as mean + SEM; n=12-13 per group. Statistical significance was determined by one-way ANOVA with Dunnett's post-test. **p<0.01, ****p<0.0001 vs 6-OHDA control.

Abbreviations: **6-OHDA**, 6-hydroxy dopamine; **AKT**, protein kinase B; **ERK**, extracellular signal-regulated kinase; **HGF**, hepatocyte growth factor; **HTRF**, homogeneous time-resolved fluorescence; **IL-1β**, interleukin-1-beta; **IL-6**, interleukin-6; **LPS**, lipopolysaccharide; **MPP+**, 1-methyl-4-phenylpyridinium; **PD**, Parkinson's disease; **PO, QD**, oral administration per day; **TNF-α**, tumor necrosis factor alpha;

References: **1.** Mhyre et. al. (2012). *Subcell Biochem*, 65, 389-455. **2.** Matsumoto K et al.(2014). *Biomedicines*, 2(4):275-300. **3**. Maina et. al. (1999) *Nat Neurosci*. 1999;2(3):213-217. **4.** Kitamura et al. (2019). *Int J. Mol. Sci*, 20(5), 1054

Acknowledgments: This study was sponsored and funded by Athira Pharma, Inc. Research support was provided by Sai Life Sciences, MD Biosciences, and Syngene International and funded by Athira Pharma Inc.