Small-Molecule HGF/MET Positive Modulators Effectively Reduce Pain-Related Behaviors in a Rat Model of Diabetic Neuropathy

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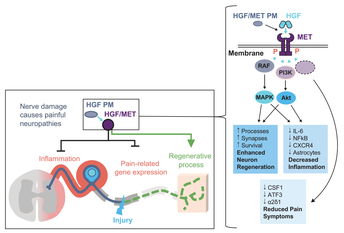
INTRODUCTION

- Roughly 60% of patients with diabetes mellitus have neuropathic pain[1]
- Neuropathic pain is largely caused by underlying damage to sensory neurons[2]
- Hepatocyte growth factor (HGF) and its receptor, MET, play a critical role in promoting neuroprotective, neurotrophic, and anti-inflammatory mechanisms[3-5]
- We have developed a platform of small molecule positive modulators of HGF/MET, two of which are ATH-1018 and ATH-1019

Figure 1. Positive Modulation of the HGF/MET Pathway Stimulates Downstream Signaling Pathways That May Provide Therapeutic Benefit in Peripheral Neuropathy



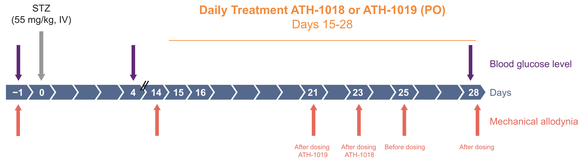
α2δ1, calcium channel subunit α2δ1; Akt, protein kinase B; ATF3, activating transcription factor 3; CSF1, macrophage colony-stimulating factor 1; CXCR4, chemokine receptor type 4; HGF, hepatocyte growth factor; IL-6, interleukin 6; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor kappa B; P, phosphorylation; PI3K, phosphoinositide 3-kinase; PM, positive modulator; RAF, rapidly accelerated fibrosarcoma.

OBJECTIVE

To evaluate the efficacy of ATH-1018 and ATH-1019 in the treatment of neuropathic pain in a rat model of streptozotocin (STZ)-induced diabetic neuropathy

METHODS

Figure 2. Experimental Timeline

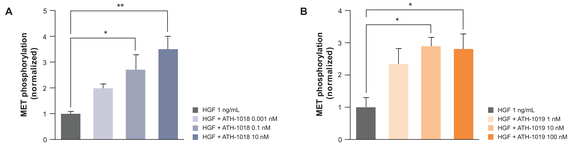


IV, intravenous; PO, by mouth; STZ, streptozotocin.

- Diabetes was induced in 6- to 8-week-old Sprague Dawley rats by a single dose of STZ (55 mg/kg intravenously [IV])
- Each study compared 4 doses of ATH-1018 (0.156, 0.625, 2.5, and 10 mg/kg by oral gavage [PO]; n = 13, each treatment group) or ATH-1019 (0.00625, 0.025, 0.1, and 1 mg/kg PO; n = 12, each treatment group)
- Each study had 3 control groups: a sham control that received vehicle rather than STZ (normal control), a diabetic neuropathic pain (DNP) control that received vehicle doses rather than drug treatment (DNP control), and a reference control group treated with pregabalin (30 mg/kg) (DNP + pregabalin)
- Mechanical allodynia was assessed using von Frey filaments (1.4, 4, 10, 15, 26, 48, and 60 g)

RESULTS

Figure 3. ATH-1018 and ATH-1019 Significantly Promoted Activation of MET In Vitro

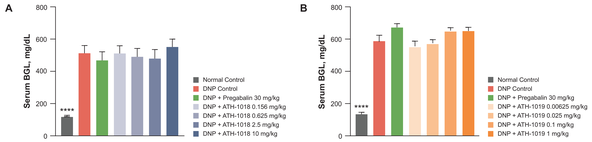


When combined with subthreshold levels of HGF, treatment with (A) ATH-1018 or (B) ATH-1019 resulted in significantly increased levels of MET phosphorylation *in vitro* (1-way ANOVA with Dunnett's multiple comparisons vs HGF 1 ng/mL).

HGF, hepatocyte growth factor.

* $P < 0.05$; ** $P < 0.01$.

Figure 4. Confirmation of Diabetic Phenotype: Elevated Blood Glucose Levels on Day 4 After STZ Injection

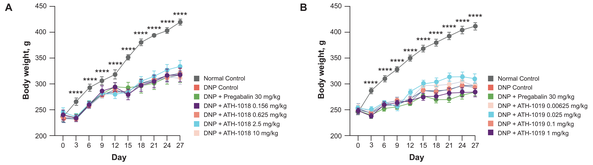


Blood glucose levels were comparable across all diabetic neuropathy groups treated with either (A) ATH-1018 or (B) ATH-1019, and the normal control group had significantly lower blood glucose levels (1-way ANOVA with Dunnett's multiple comparisons vs DNP control).

BGL, blood glucose level; DNP, diabetic neuropathic pain; STZ, streptozotocin.

**** $P < 0.0001$.

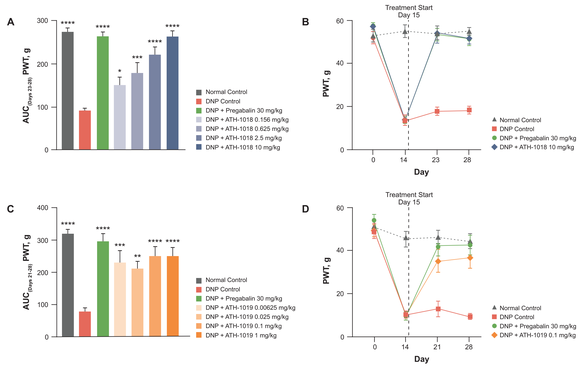
Figure 5. Diabetic Animals Had Significantly Lower Body Weights Compared to Normal Control Animals



Nondiabetic normal controls had significantly higher body weights than DNP controls; no differences in body weights were noted between any of the diabetic groups receiving either (A) ATH-1018 or (B) ATH-1019 (2-way ANOVA with Dunnett's multiple comparisons vs DNP control).

**** $P < 0.0001$.

Figure 6. Treatment With ATH-1018 or ATH-1019 Significantly Reduced Mechanical Allodynia



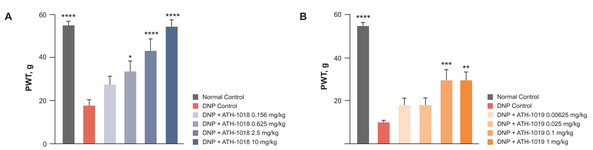
(A) Animals treated with ATH-1018 exhibited a dose-dependent response (1-way ANOVA with Dunnett's multiple comparisons vs DNP control). (B) PWTs in animals treated with 10 mg/kg ATH-1018 were increased over diabetic controls by day 23. (C) Animals treated with ATH-1019 had significantly increased PWTs (1-way ANOVA with Dunnett's multiple comparisons vs DNP control). (D) PWTs in animals treated with 0.1 mg/kg of ATH-1019 were increased over diabetic controls by day 21.

AUC, area under the curve; DNP, diabetic neuropathic pain; PWT, paw withdrawal threshold.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

- Across all tested doses of ATH-1018 and ATH-1019, a significant decrease in mechanical allodynia was seen, as indicated by increased paw withdrawal thresholds (PWTs) across the treatment period
- Treatment with ATH-1018 elicited a dose-dependent response, with higher doses exhibiting a highly significant increase in PWT area under curve (AUC) over the treatment period, and dosing with 10 mg/kg resulting in significant increase by day 23
- Improvement in PWT AUC over the treatment period was robust across tested doses of ATH-1019, with no clearly identifiable dose-dependent response, and treatment with ATH-1019 resulted in a significant increase in PWTs by day 21

Figure 7. Reduction in Mechanical Allodynia Persisted After Clearance of ATH-1018 and ATH-1019



(A) PWTs in animals treated with ATH-1018 were significantly increased compared with diabetic controls (1-way ANOVA with Dunnett's multiple comparisons vs DNP control). (B) PWTs in animal treated with ATH-1019 at higher doses were significantly increased compared with diabetic controls (1-way ANOVA with Dunnett's multiple comparisons vs DNP control).

DNP, diabetic neuropathic pain; PWT, paw withdrawal threshold.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

- Pain was assessed on day 25 before dosing
- Both ATH-1018 and ATH-1019 are rapidly cleared from blood plasma, with $t_{1/2}$ being 0.5 to 1 hour (complete clearance in 3.5-7 hours), allowing PWT to be assessed before dosing to evaluate persistent effects
  - Because pregabalin has a $t_{1/2}$ of 6.3 hours (complete clearance in 44 hours), it was still present in blood plasma at the time of assessment, and PWT was not assessed for this control group
- PWT was significantly higher in animals treated with ATH-1018, with a clear dose-dependent response, even when mechanical allodynia was assessed before dosing
- Before dosing, animals given higher concentrations of ATH-1019 had significantly higher PWTs

References

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Acknowledgments

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Disclosures

Jewel Johnston, Andrée-Anne Berthiaume, Robert Taylor, and Kevin Church are employees and stockholders of Athira Pharma, Inc.

CONCLUSIONS

- Daily oral treatment with ATH-1018 or ATH-1019 in rats with STZ-induced diabetic neuropathy significantly reduced pain responses
  - Animals treated with ATH-1018 showed significant inhibition of mechanical allodynia
    - All tested doses produced significantly increased PWTs, with a clear dose-dependent response
  - Animals treated with ATH-1019 showed significant inhibition of mechanical allodynia
    - All tested doses produced significantly increased PWTs
- At day 25 (treatment day 10), animals showed significant inhibition of mechanical allodynia before receiving treatment with ATH-1018 or ATH-1019, suggesting that the compounds may have lasting disease-modifying effects, even once cleared from circulation

Based on these promising preclinical results, ATH-1018 and ATH-1019 are being developed as potential treatments for diabetic neuropathy and other neurological disorders

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Title: Small-Molecule HGF/MET Positive Modulators Effectively Reduce Pain-Related Behaviors in a Rat Model of Diabetic Neuropathy
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Methods

Validation of HGF/MET Phosphorylation

- HEK293 cells were treated with HGF 1 ng/mL and varying concentrations of ATH-1018 or ATH-1019
  - HGF at 1 ng/mL was empirically determined as a subthreshold dose of HGF, which does not significantly induce MET phosphorylation (pMET)
- Enzyme-linked immunosorbent assay (ELISA) was used to quantify levels of pMET

Study Design

- On day 0, diabetes was induced in 6- to 8-week-old Sprague Dawley rats by a single dose of STZ at a concentration of 55 mg/kg intravenously (IV)
- On day 4, rats were screened for serum blood glucose levels >250 mg/dL to confirm diabetes
- Neuropathic pain-related behaviors were characterized on day 14
- ATH-1018 and ATH-1019 were investigated in 2 separate studies with similar study protocols
  - Each study had 3 control groups:
    - The sham control group received vehicle rather than an STZ injection on day 0 (n = 12)
    - The diabetic control group received vehicle injections rather than drug treatment (n = 12)
    - The positive control group was treated with pregabalin (10 mL/kg) (n = 12-13)
  - ATH-1018 experimental groups received 4 doses (0.156, 0.625, 2.5, and 10 mg/kg by mouth [PO]) once daily (n = 13, each treatment group)
  - ATH-1019 experimental groups received 4 doses (0.00625, 0.025, 0.1, and 1 mg/kg PO) once daily (n = 12, each treatment group)
  - Treatments were administered from days 15-28

In Vivo Pain Assay

- Mechanical allodynia was assessed using von Frey filaments (1.4, 4, 10, 15, 26, 48, and 60 g)
  - Filaments were pushed against the animal's foot, and the paw withdrawal threshold (PWT, in grams) was established as the force that resulted in a pain response
  - In the ATH-1018 study, behaviors were assessed 1 hour after dosing on days 23* and 28 (treatment days 9 and 14)

    *Study design planned for assessment on day 21 instead of 23, but research organization ran into COVID-related staffing issues
  - In the ATH-1019 study, behaviors were assessed 1 hour after dosing on days 21 and 28 (treatment days 7 and 14)
  - On day 25, there was an additional evaluation before treatment (treatment day 10, after the 9th dose had cleared from the blood plasma [at >7× the half-life ($t_{½}$)])

Statistical Analyses

- Statistical analyses were performed using Prism 9 (GraphPad, Inc.)
  - Mean body weight for control and treatment groups was assessed using a two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test versus the diabetic control group
  - Blood glucose level and PWT metrics for mechanical allodynia were assessed using a one-way ANOVA with Dunnett's multiple comparison test versus the diabetic control group