The Effect of ANG-3777 on *In Vitro* Cell Proliferation

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Background

Hepatocyte growth factor (HGF) is an endogenous protein that is released in response to organ damage. HGF is the natural ligand of the c-Met receptor, which is also increased in response to injury[1]. The biological effects of HGF stimulation of c-Met leads to activation of cellular pathways that results in cell survival and cellular proliferation[2,3]. These processes are activated after HGF binds to c-Met which triggers the dimerization and phosphorylation of the receptor (Fig. 1). ANG-3777 is a small molecule mimetic of HGF. It has been demonstrated to exert similar cytoprotective and regenerative effects as HGF.[4] The objective of this study was to determine whether ANG-3777 recapitulates the activity of HGF in stimulating cell proliferation in vitro by evaluating the proliferative effect of ANG-3777 on human umbilical vein endothelial cells (HUVECs), rat neuronal Schwann cells, and mouse fibroblasts by utilizing [3H]-thymidine incorporation assays.

Methods

ANG-3777 and recombinant Human HGF Protein (R&D Systems) was dissolved in dimethyl sulfoxide (DMSO). DMSO was used as the vehicle control; the final DMSO concentration in cell culture was 0.1%.

HUVEC Cells: HUVECs were seeded at 1000 cells per well in 48-well plates in medium containing 2% serum and growth supplements and grown overnight to 30% to 40% confluency in serum-containing complete medium. They were then serum-starved for 1 to 2 hours in Roswell Park Memorial Institute (RPMI) medium containing 1% bovine serum albumin (BSA), followed with treatment with HGF (25 ng/mL), ANG-3777 (5 µM), or DMSO (vehicle), overnight.

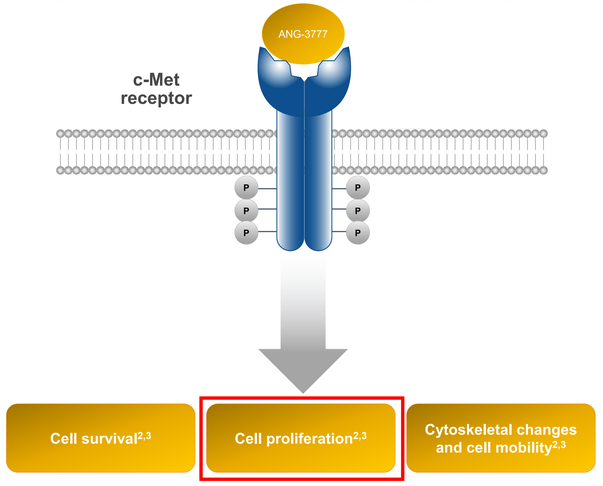
Schwann Cells: RSC96, a rat Schwann cell line, were seeded at 1000 cells per well in 96-well until the culture reached 30% to 40% confluency, within 20 to 48 hours. Cells were then serum-starved for 1 to 2 hours in RPMI medium containing 1% BSA, followed by treatment with HGF (3 to 100 ng/ml), ANG-3777 (0.01 to 10 µM), or DMSO (vehicle) in triplicate wells for each concentration, and incubated for 16 to 24 hours.

Fibroblasts: NIH/3T3 cells, a mouse fibroblast cell line, were seeded at 1000 to 1500 cells per well in 48-well plates in complete medium containing 10% serum and grown to 30 to 40% confluency for 24 hours. Cells were then serum-starved for 1 to 2 hours in RPMI medium containing 1% BSA, followed by treatment with HGF (25 ng/ml), ANG-3777 (17 µM), or DMSO for 16 to 24 hours.

Proliferation Assessment: [3H]-thymidine was added at 10 µCi/mL to the medium and incubation of each cell line continued for another 4 to 5 hours. Cells were washed 3 times with PBS and lysed with 0.5 mL of alkaline lysis buffer containing 0.5 N NaOH and 1 % sodium dodecyl sulfate (SDS). After incubation on a shaker for 1 hour at room temperature, the cell lysates were placed in scintillation vials to which 3.5 mL of scintillation fluid was added. The [3H] signal, representing thymidine incorporation into to newly synthesized DNA (a measure of cell proliferation), was counted using a beta counter.

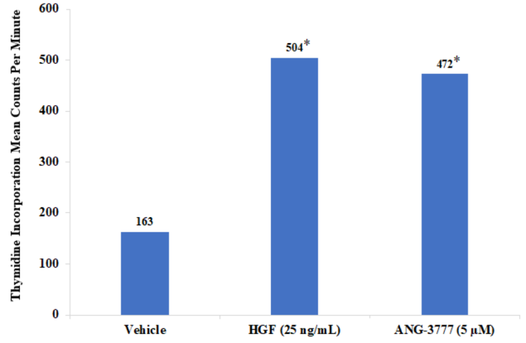
Results

Figure 1: c-MET receptor signaling pathway



HUVEC Cells: ANG-3777, like HGF, increased [3H]-thymidine incorporation in HUVECs, indicating ANG-3777 has a similar activity as HGF stimulating HUVEC proliferation (Figure 2).

Figure 2: Effect of ANG-3777 vs HGF on HUVEC Cell Proliferation

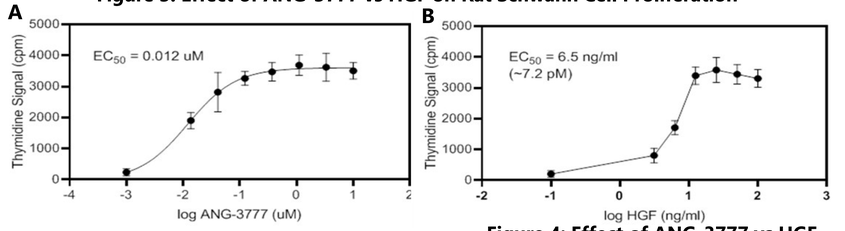


*p<0.01 vs vehicle

Results (2)

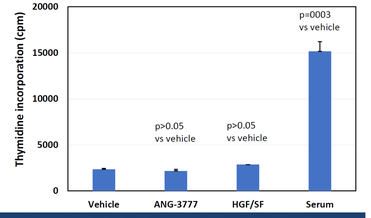
Schwann Cells: ANG-3777 and HGF increased [3H]-thymidine incorporation in a concentration–dependent manner (Fig. 3). The effective concentration at 50% proliferative activity (EC_{50}) of each was determined as indicated in Figure 3 (A. ANG-3777; B. HGF).

Figure 3: Effect of ANG-3777 vs HGF on Rat Schwann Cell Proliferation



Fibroblasts: In NIH/3T3 fibroblasts, which do not express the c-Met receptor, neither HGF nor ANG-3777 stimulated cell proliferation (Figure 4). As a positive control, serum, which contains many growth stimulating factors, was evaluated in parallel and was found to stimulate NIH/3T3 cell proliferation (Figure 4).

Figure 4: Effect of ANG-3777 vs HGF on Fibroblast Proliferation



Conclusion

In vitro, ANG-3777:

1. Mimics the effect of HGF and induces proliferation in c-Met-receptor-expressing human endothelial and rat Schwann cells
2. In fibroblasts, which lack c-Met, the proliferative effects were absent in a similar manner as HGF.

These results demonstrate that ANG-3777 induces proliferation in a comparable manner to HGF and that this effect is dependent on the c-Met receptor.

References

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