In Vivo Phosphorylation of c-MET receptor by ANG-3777, a hepatocyte growth factor mimetic

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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 demonstrated to exert similar cytoprotective and regenerative effects as HGF[4]. The objectives of these studies were to evaluate *in vivo* the interaction of ANG-3777 with c-Met to stimulate phosphorylation of c-Met, which would lead to the activation of intracellular reparative pathways (Fig. 1). Study 1 assessed the ability of ANG-3777 to stimulate the phosphorylation of the c-Met receptor in the rat liver. Study 2 assessed the ability of ANG-3777 to induce phosphorylation in the rat kidney in a model of renal ischemia and reperfusion injury.

Methods

Studies were conducted following an approved Institutional Animal Care and Use Committee (IACUC) protocol.

Animals. Adult male Sprague-Dawley (SD) rats (300 g) purchased from Charles River Laboratories.

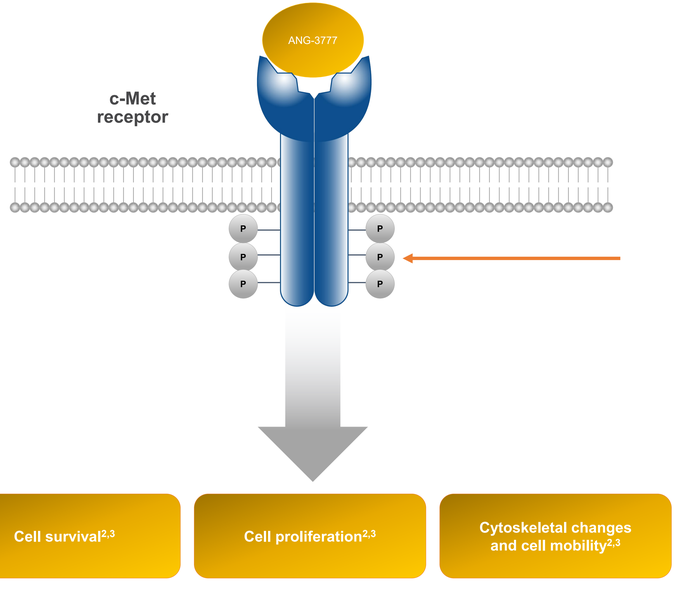
Treatments. Study 1: 0.5 ml of 24 mg/ml solution of ANG-3777 in dimethyl sulfoxide (DMSO) administered intraperitoneal (IP). Study 2, 0.2 ml of a 3 mg/ml solution of ANG-3777 in DMSO was given intravenously (IV). In both studies, vehicle was DMSO.

Study 1. SD rats were treated with 40 mg/kg ANG-3777 or vehicle via intraperitoneal (IP) injection. Animals were sacrificed at 0 minute, 30 minutes, 1 hour, and 2-hour time points and perfused with PBS for 5 minutes. Liver was collected and weighed. The liver samples were normalized by weight and homogenized. The sample aliquots were diluted in RIPA buffer and analyzed by Western blot using rabbit phospho-Met antibody.

Study 2. SD rats were subjected to 60-minute normothermic unilateral ischemia followed by 24-hour reperfusion. At the onset of reperfusion, the contralateral kidney was excised. ANG-3777 (2 mg/kg, intravenously [IV]) or vehicle was administered 18 hours after reperfusion was initiated. Animals were sacrificed at 18, 20, or 24 hours of reperfusion (0, 2, and 6 hours after treatment) and total c-Met and phosphorylated c-Met in kidney extracts were assessed by Western blot analysis.

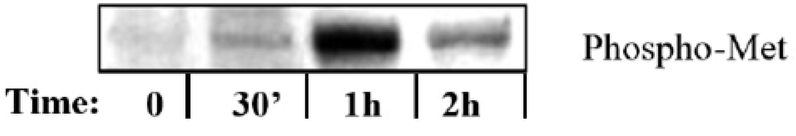
Results

Figure 1: c-MET receptor signaling pathway



Study 1: c-Met phosphorylation was time dependent. ANG-3777 phosphorylated c-Met in the liver with peak intensity at 1-hour post injection

Figure 2: *In Vivo* c-Met phosphorylation Kinetics in Liver

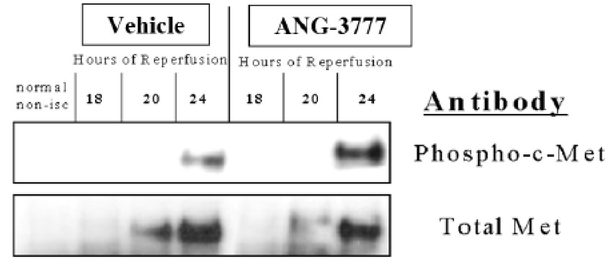


Phospho=phosphorylated

Results (2)

Study 2: Ischemia-reperfusion injury to the kidney provoked a time-dependent increase in total c-Met. ANG-3777 and vehicle treated cohorts resulted in a peak phosphorylation of c-MET at 24 hours (6-hours post ANG-3777 treatment) and no phosphorylation at 18 and 20-hours post reperfusion (0 and 2-hours post ANG-3777 treatment).

Figure 3: ANG-3777 Phosphorylates Kidney c-Met receptor



Non-isc=non-ischemic; Phospho=phosphorylated

Conclusion

In SD rats, ANG-3777 induced:

1. Phosphorylation of the c-Met receptor in the liver in a time-dependent manner, with peak levels at one hour post-injection
2. A robust increase in phosphorylated c-Met at 24 hours post-reperfusion (6 hours post ANG-3777 treatment) in a rat renal ischemia and reperfusion model

These results demonstrate that ANG-3777 administration induces c-Met phosphorylation *in vivo*. Clinical research is ongoing to evaluate if these effects may translate into a treatment of acute kidney injury (AKI) in humans.

References

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