Analgesic properties of a novel brain-penetrant Angiopep-2-Neurotensin derivative (ANG2002) for treating chronic pain

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ABSTRACT

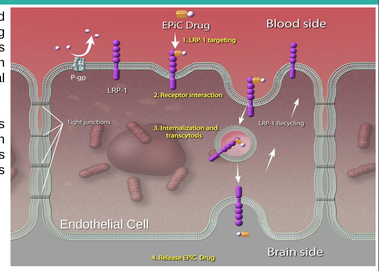
The blood-brain barrier (BBB) consists of brain capillary endothelial cells that are connected by tight junctions and express high levels of active efflux transport proteins. Restricting entry of xenobiotics into the brain, the BBB poses a challenge to CNS drug discovery. Essential molecules such as nutrients and hormones are transported across the BBB via proteins such as the low-density lipoprotein related receptor (LRP1). Based on sequences from LRP1 ligands, a family of peptides (Angiopeps) has been designed to confer BBB permeability (Engineered Peptide Compound technology, EPiC).

In the present study, we have designed a new chemical entity by conjugating the Angiopep-2 (ANG) peptide with the 13 amino acid neuropeptide, neurotensin (NT). While not centrally active following systemic delivery, NT produces strong analgesia when administered directly into the brain. Using a mouse in vivo paradigm, we show that the ANG-NT (ANG2002) derivative is transported at least 10-fold more efficiently across the BBB than native NT. In vitro, ANG2002 binds to both NT receptors (NTS1 and NTS2) with affinities similar to those of native NT. In addition, in vivo studies demonstrate that ANG2002 induces long-lasting analgesia in distinct pain models. In rats, ANG2002 induces a dose-dependent analgesia in both phases of the formalin model of persistent pain. The median effective analgesic iv doses (ED50) of ANG2002 are 0.009 and 0.016 mg/kg for the acute and inflammatory phases, respectively. At a dose of 0.05 mg/kg, ANG2002 was also effective in reversing pain behaviors induced by chronic constriction injury of the sciatic nerve (neuropathic pain) or femoral inoculation of MRMT-1 rat breast cancer cells (bone cancer pain).

The analgesic properties of this new brain-penetrant ANG-NT derivative (ANG2002) suggest that this conjugate may be effective for clinical management of acute and chronic pain. These data extend the applicability of Angiopep-2 conjugation from small molecules to peptides such as neurotensin, and further establish the benefits of Angiochem's technology for the development of new CNS therapeutics.

INTRODUCTION

Angiochem is a clinical-stage biotechnology company discovering and developing new breakthrough drugs that are uniquely capable of crossing the blood brain barrier (BBB) to treat brain diseases. These new drugs have the potential to address significant medical needs, many of which cannot be effectively addressed due to the fundamental physiological challenge the BBB presents.



The BBB is a selective barrier formed by tightly packed endothelial cells that line the cerebral capillaries. The BBB is important as it provides an insulated environment for stable neuronal function. Endothelial cells forming the BBB are not only able to form tight junctions, but also possess the following characteristics that further protect the brain, they:

- ✓Lack fenestra;
- ✓Lack transendothelial channels;
- ✓Lack pinocytic vesicles; and
- ✓Express high levels of the active efflux pump (P-gp).

Angiochem's proprietary peptide-drug conjugate platform targets the low-density lipoprotein receptor-related protein (LRP) receptor family. This endogenous transcytosis system has a number of inherent biochemical advantages for drug transport across the BBB, including high capacity, rapid turnover, recognition of numerous ligands, and limited down-regulation. We have created peptides (Angiopeps), including Angiopep-2 (AN2) using a library based on LPR-1 binding sequences of known LRP-1 ligands. These peptides can be introduced, by chemical conjugation or fusion, to small molecules and biologics.

Neurotensin (NT) is a 13 aa peptide that has been associated with multiple functions in brain. One such function is pain sensation, however, as NT does not cross the BBB effectively, this effect is not observed unless NT is administered icv. Chemical conjugation of AN2 to NT results in a 32 aa peptide that can cross the BBB to achieve therapeutic levels in brain. This novel peptide-drug conjugate, ANG2002, was tested in multiple rodent models of pain sensation and found to be efficacious at low doses when administered iv.

Angiopep-2 Thr-Phe-Phe-Tyr-Gly-Gly-Ser-Arg-Gly-Lys-Arg-Asn-Asn-Phe-Lys-Thr-Glu-Glu-Tyr-NH_2
LRP-1 receptor binding site

ANG2002 structure

Neurotensin pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH
NT receptor binding site

METHODS

1. Evaluation of in vivo brain uptake:

Test drug (^{125}I-labeled) was administered via carotid artery of mice for up to 4 minutes, followed by a 30-second saline wash. Brains were removed followed by capillary depletion, radioactivity quantification, and calculation of rate constant, Kin.

2. Evaluation of analgesic effect in pain models:

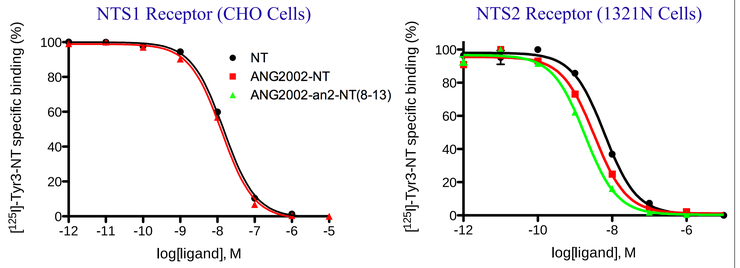
Hot plate mouse model: Mice were placed onto a hot metal plate maintained at 54ºC and foot-licking response was measured after dosing. Latency to a hindlimb response (lick, shake or jump) was recorded, with a maximum time on the hot plate of 30 seconds.

Rat formalin induced pain model: Rats were dosed iv with test drug or vehicle 10 minutes prior to receiving a unilateral intraplantar injection of formalin. Beginning 5 minutes later, pain-related behaviors were assessed for a total of 60 minutes. Pain behavior was scored based on the time spent lifting, shaking, biting, scratching, or licking the affected paw during 3-minute intervals. A statistically significant increase from baseline pain-threshold measurement was interpreted as induction of analgesia.

Rat chronic constriction injury (CCI) model: Ligatures were applied to the sciatic nerves of adult male SD rats. Onset of neuropathy was evaluated using automatic von Frey testing on days 0, 7, 20, and 28. Results are expressed as paw withdrawal threshold (PWT) in grams (g). On day 28, rats were given iv administration of ANG2002 (0.05 mg/kg) and the anti-allodynic effect was monitored at different post-treatment time points. A statistically significant increase from baseline pain-threshold measurement was interpreted as induction of analgesia.

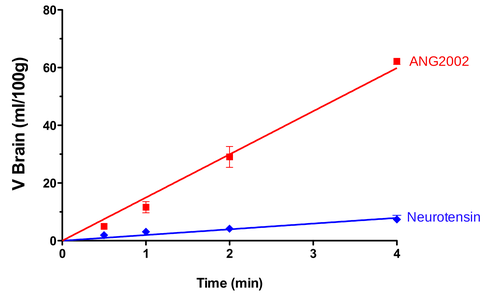
PHARMACOLOGY OF ANG2002, NT DERIVATIVE

^{125}I Neurotensin Competition Binding



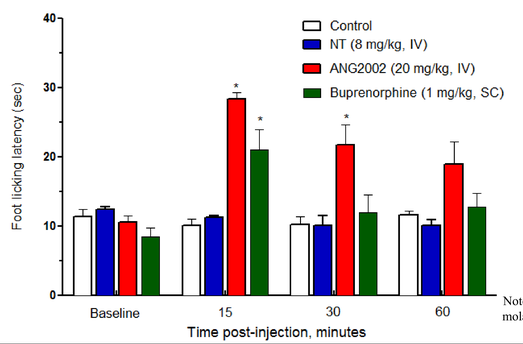
Compound	h NTS1 (IC_{50} nM)	h NTS2 (IC_{50} nM)
NT	4.0	4.0
ANG2002	13.0	3.3
ANG-NT(8-13)	NA	1.8

BRAIN UPTAKE OF ANG2002 IN MOUSE



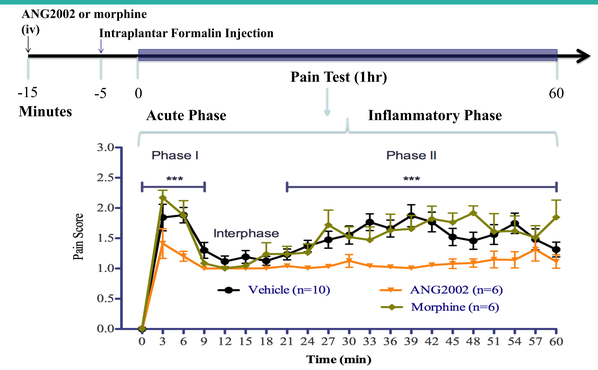
Molecule	K_{in} (mL/s/g)	Ratio
NT	2.5 x 10^{-4}	1
ANG2002	2.7 x 10^{-3}	11

ANG2002 ANALGESIC EFFICACY: MOUSE HOT PLATE

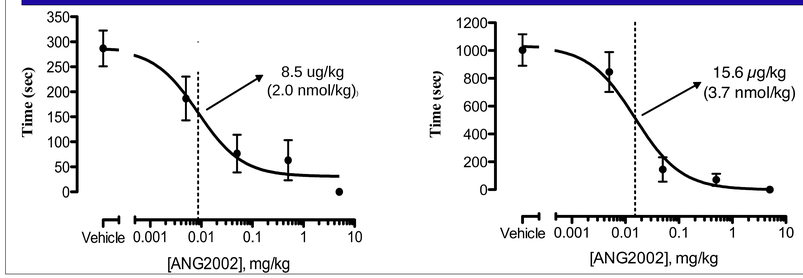


Note: 20 mg/kg ANG2002 is the molar equivalent of 8 mg/kg NT.
*=p<0.05

ANG2002 EFFICACY: RAT FORMALIN-INDUCED PAIN MODEL

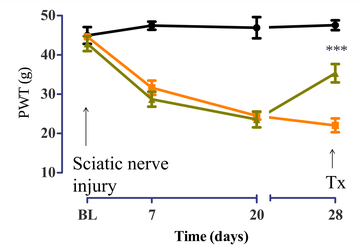


ED_{50} for licking, biting, & shaking: Phase I **Phase II**

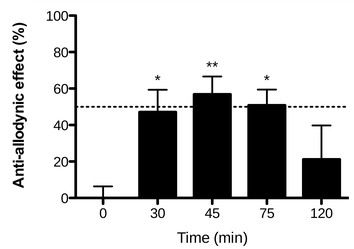


ANG2002 EFFICACY: RAT CHRONIC CONSTRICTION INJURY

Paw Withdrawal Threshold Following Injury



ANG2002 (0.05 mg/kg) increases PWT



CONCLUSIONS

- A new, high affinity, brain-penetrant NT agonist peptide-drug conjugate (ANG2002) is described.
- While native NT is ineffective due to poor brain penetration, ANG2002 shows analgesic effects in the mouse hot plate assay, increasing foot-licking latency by over two fold compared to control.
- ANG2002 is effective in the rat formalin paw test, reducing pain score with ED_{50} values of 8.5 μg/kg and 15.6 μg/kg, in the acute and inflammatory phases, respectively.
- ANG2002 reduces allodynia in the rat chronic constriction injury model at 0.05 mg/kg.
- These results validate the use of AN2 Peptide-drug conjugates to create brain-penetrant neuro-peptides, expanding the potential utility to other neuropeptides for pain and other CNS disorders.

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