ANG2002: a new Angiochem-modified neurotensin with increased brain penetration and analgesic properties

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ABSTRACT

The blood-brain barrier (BBB) is mainly formed by brain capillary endothelial cells which are closely sealed by tight junctions and express high levels of active efflux transport proteins. As a result of these restrictions, approximately 98% of small molecules and nearly 100% of large molecules, such as recombinant proteins or gene-based medicines do not cross the BBB. Overcoming the obstacle posed by the BBB is, therefore, a critical goal of CNS drug development and therapy.

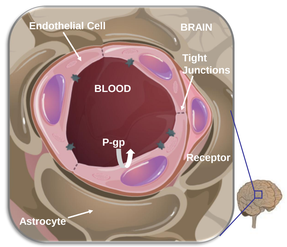
A new family of peptides derived from proteins that efficiently cross the BBB using low-density lipoprotein receptor-related protein (LRP) has been designed and is incorporated in new therapeutics for uptake into the brain. This new engineered peptide compound platform technology (EPiC) is applicable to small and large molecules and provides a non-invasive and flexible platform creating new drugs which have access to the central nervous system using LRP for the treatment of CNS diseases. Based on these properties, we have created a portfolio of new drug entities composed of small molecules, peptides and mAbs, the most advanced of which is ANG1005 formed by chemical conjugation of our peptide to three molecules of paclitaxel. ANG1005 demonstrated safety and efficacy in two phase ½ clinical trials for the treatment of primary and secondary brain tumors in humans.

In the present study, we have investigated the brain uptake of a new chemical entity formed by conjugation of the peptide Angiopep-2 (ANG) and neurotensin. Here, we show by mice *in-situ* brain perfusion that the ANG-Neurotensin (ANG2002) is transported very efficiently across the BBB. The transport rate is higher than that of unconjugated neurotensin by at least 10-fold. *In vitro*, ANG2002 is able to bind to the high affinity neurotensin receptor. In addition, *in vivo* studies demonstrated that ANG2002 induces analgesia in acute and inflammatory pain models.

In conclusion, these data confirm that in addition to small molecules, conjugation of neuropeptide such as neurotensin to Angiopep-2 significantly enhances their entry into the brain. This further validates the use of Angiochem's technology for new therapies of CNS disorders or diseases.

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.



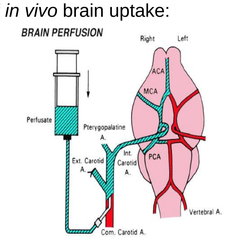
The BBB is a selective barrier formed by tightly packed endothelial cells that line the cerebral capillaries. The BBB is important as it provides an insulated environment for stable neuronal function. Endothelial cells forming the BBB are not only able to form tight junctions, but also possess the following 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 EPiC 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 expression, rapid turnover, numerous ligands of varying sizes, and limited down-regulation. Neurotensin (NT), an endogenous tridecapeptide that induces antinociception and hypothermia in the central nervous system, is of potential use for the treatment of neuropathic pain, as well as other pain syndromes. However, because NT penetrates poorly through the blood-brain barrier (BBB), its potential as a therapeutic agent has been difficult to realize. In the present study, we investigated the brain uptake and analgesic effects of a new chemical entity formed by conjugation of NT to Angiopep-2, a 19-mer peptide that crosses the BBB. Results of *in-situ* brain perfusions in mice demonstrated that the Angiopep-2-NT conjugate, ANG2002, efficiently penetrated the blood-brain barrier with a transport rate at least 10-fold higher than that of unconjugated NT. Importantly, ANG2002 exhibited activity in various animal pain models including neuropathic pain, encouraging its development as a therapeutic agent for this later difficult-to-treat pain syndrome as well as for post-operative or cancer pain. At a more general level, the isolation of ANG2002 further demonstrates the potential of the EPiC platform for the development of neurotherapeutics with enhanced brain penetration. Currently, 4 EPiC agents, including ANG2002, have been isolated and characterized, all of which exhibit rapid transcytosis across the BBB and subsequent biological activity. It is hoped that this platform will continue to be a valuable source of other therapeutics for CNS disorders and diseases in the future.

METHODS

1. Evaluation of *in vivo* brain uptake:



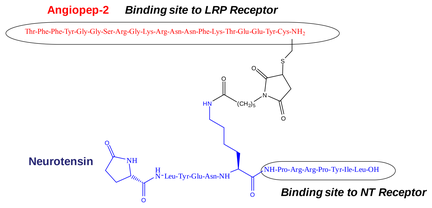
Animals: mice
Perfusion in the right carotid artery
Perfusion time: 0-10 min
Perfusion rate: 1.15 ml/min
Radiotracers: ^{125}I-ANG2002
^{125}I-NT
Washout with saline: 30s
Quantification of radioactivity in the brain

2. Evaluation of analgesic effect in pain models:

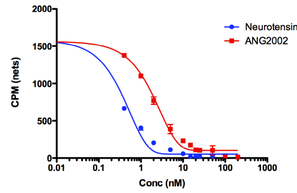
- **Hot plate mouse assay**: Mice were placed onto a hot metal plate maintained at 54ºC and foot-licking response was measured after dosing.
- **Formalin-induced pain mouse model**: Analgesic activity was assessed by recording the hind paw licking time recorded at 5-minute intervals during the 35-minute period after formalin subplantar injection (0.02 ml, 2% solution).
- **Brennan post-operative rat model**: A 1-cm longitudinal incision was made in the plantar of the foot. Evaluations for mechanical allodynia were performed on day 1 post-surgery (pre-dosing baseline). On day 2 post-surgery, the rats were assessed again for responses to von Frey mechanical allodynia testing after dosing.
- **Rat Chung model**: 14 days after the ligation of the spinal nerves, response to mechanical allodynia was evaluated (pre-dosing). On day 18, animals were re-tested for mechanical allodynia after dosing.

NEW NEUROTENSIN (ANG2002) DERIVATIVE

A. ANG2002 chemical structure



B. Competition [^{3}H]-neurotensin binding assay on HT29 cells

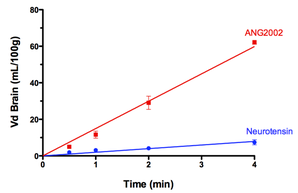


IC50 values	(nM)
Neurotensin	0.4
ANG2002	1.7

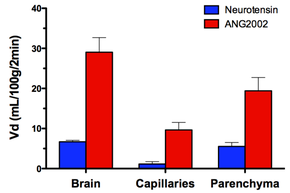
A.Chemical structure of the Angiopep-2-Neurotensin conjugate (ANG2002). B. Competition binding [^{3}H]-neurotensin assay on HT29 cells. HT-29 cell monolayers were incubated with [^{3}H]-neurotensin (0.4 nM) and increasing concentrations (0 to 200 nM) of unlabeled neurotensin or unlabeled ANG2002 for 30 minutes at 37 °C in binding buffer. Binding inhibition results were analyzed using GraphPad Prism software.

BRAIN UPTAKE OF ANG2002

A. *In situ* brain perfusion



B. Brain compartment distribution of ANG2002 after brain capillary depletion



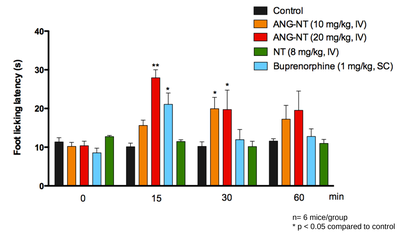
C. Initial brain transport rates (Kin)

Drug	Brain K_{in} (mL/s/g)
Glucose	9.5 x 10^{-3}
ANG1005 (Angiopep-2-Paclitaxel)	7.3 x 10^{-3}
ANG1007 (Angiopep-2-Doxorubicin)	3.7 x 10^{-3}
ANG2002 (Angiopep-2-Neurotensin)	2.7 x 10^{-3}
ANG2006 (Angiopep-2-Leptin)	1.2 x 10^{-3}
Neurotensin	2.3 x 10^{-4}
Alcohol	1.8 x 10^{-4}
Morphine	1.6 x 10^{-4}
Insulin Rec Antibody	1.0 x 10^{-4}
Paclitaxel and Doxorubicin	~5 x 10^{-5}
RAP	1.0 x 10^{-5}
mAb	~6 x 10^{-5}

***In vivo* brain uptake of the [^{125}I]-ANG2002 and [^{125}I]-neurotensin was measured by *in situ* brain perfusion. B. Brain capillary depletion was performed to assess the ANG2002 distribution in the brain compartments. C. Initial brain transport rate (Kin) values for ANG2002 and neurotensin compared to that of other molecules.**

ANALGESIC PROPERTIES OF ANG2002

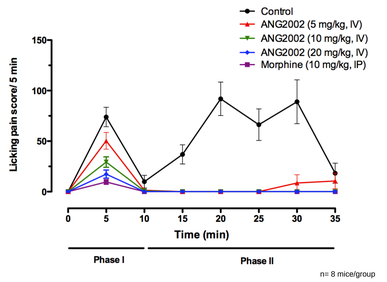
Hot plate assay



n= 6 mice/group
* p < 0.05 compared to control

Effect of ANG2002 in the mouse hot plate assay. ANG2002 significantly increased the foot licking latency whereas neurotensin (NT) does not. Buprenorphine was used as a positive control.

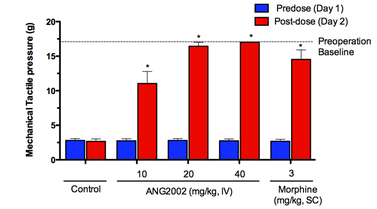
Formalin-induced pain model



n= 8 mice/group

Efficacy in the mouse formalin-induced pain model. ANG2002 significantly reduces licking responses in both phases I and II in a dose-dependent manner.

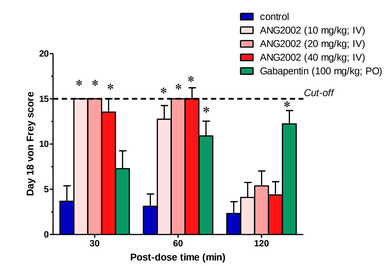
Brennan post-operative pain model



N.B. in term of molar equivalent, morphine at 3 mg/kg corresponds to about 40 mg/kg of ANG2002
n= 8 rats/group
* p < 0.05 compared to pre-dosing evaluation

Efficacy of ANG2002 in the Brennan post-operative rat model. ANG2002 significantly reduces mechanical allodynia 2 days post-surgery and 30 min after dosing.

Chung model for neuropathic pain



n= 8 rats/group
* p < 0.05 compared to control

Efficacy in the rat Chung Model . ANG2002 significantly reduces mechanical allodynia in the spinal nerve ligation model of neuropathic pain.

CONCLUSIONS

- EPiC platform for small molecules has been validated in human with our anticancer agent ANG1005 which shows promising results in Phase 1/2 studies for brain tumors.
- In the present study, the main objective was to broaden the EPiC platform for neuropeptides by generating a new Angiopep-2-neurotensin derivative (ANG2002) for pain.
- ANG2002:
  - Has a better brain penetration than native neurotensin
  - Binds to neurotensin receptors
  - Shows *in vivo* efficacy in various animal pain models:
    Acute, inflammatory, post-operative and neuropathic pain
- Strong validation of the EPiC platform for neuropeptides and opens new avenues for other potential compounds that do not cross the BBB.

angiochem
Creating drugs to treat brain diseases