ANG2002: A new brain-penetrating Angiopep-Neurotensin for the treatment of pain

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ABSTRACT

Keywords: **Antinociception • Analgesia • ANG2002 • Blood-brain barrier • Neurotensin**

Overcoming the obstacle posed by the blood-brain barrier (BBB) is a critical goal of CNS drug development and therapy. A new family of peptides developed by Angiochem that efficiently crosses the BBB using low-density lipoprotein receptor-related proteins (LRP) and provides a non-invasive and flexible platform for creating new drugs could be a major breakthrough in this field.

Neurotensin (NT), an endogenous peptide that induces antinociception in the CNS, is of potential use for the treatment of pain. However, the BBB renders its systemic injection ineffective, requiring a direct administration into the CNS. To overcome this issue, ANG2002, a novel NT-Angiopep-2 conjugate, was designed using Angiochem's platform. A carotid artery brain perfusion technique in mice demonstrates that ANG2002 is transported across the BBB ten times more efficiently than unconjugated Neurotensin. *In vitro* studies have shown that ANG2002 binds to the high-affinity Neurotensin receptor, while *in vivo* studies demonstrated that ANG2002 induces analgesia in acute and inflammatory pain models.

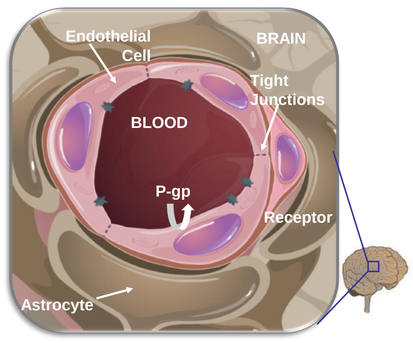
Overall, the data confirm that, in addition to small molecules, conjugating large neuropeptides such as Neurotensin to Angiopep-2 significantly enhances their entry into the brain. This further validates the use of Angiochem's peptide-based technology for new therapies of CNS disorders or diseases.

INTRODUCTION

The blood-brain barrier (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 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).

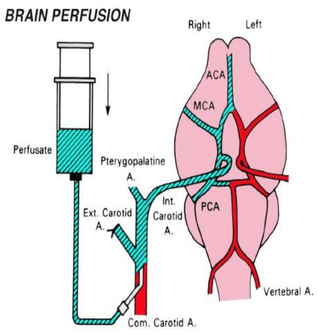
As a result of these restrictions, approximatively 98% of small and nearly 100% of large molecules do not reach the brain.



Angiochem's peptide platform targets the low-density lipoprotein receptor-related protein (LRP1), a member of the LDL-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 formalin-induced inflammatory pain model, encouraging its development as a therapeutic agent for the treatment of pain. Overall, these data extend the validation of Angiopep-2 conjugation to include neuropeptides such as NT, and further establish the benefits of this technology for the development of new neurotherapeutics.

METHODS

1. Evaluation of *in vivo brain uptake:*



Animals: mice
Perfusion in the right artery carotid
Perfusion time: 0-10 min
Radiotracers: ^{125}I-ANG2002
^{125}I-NT

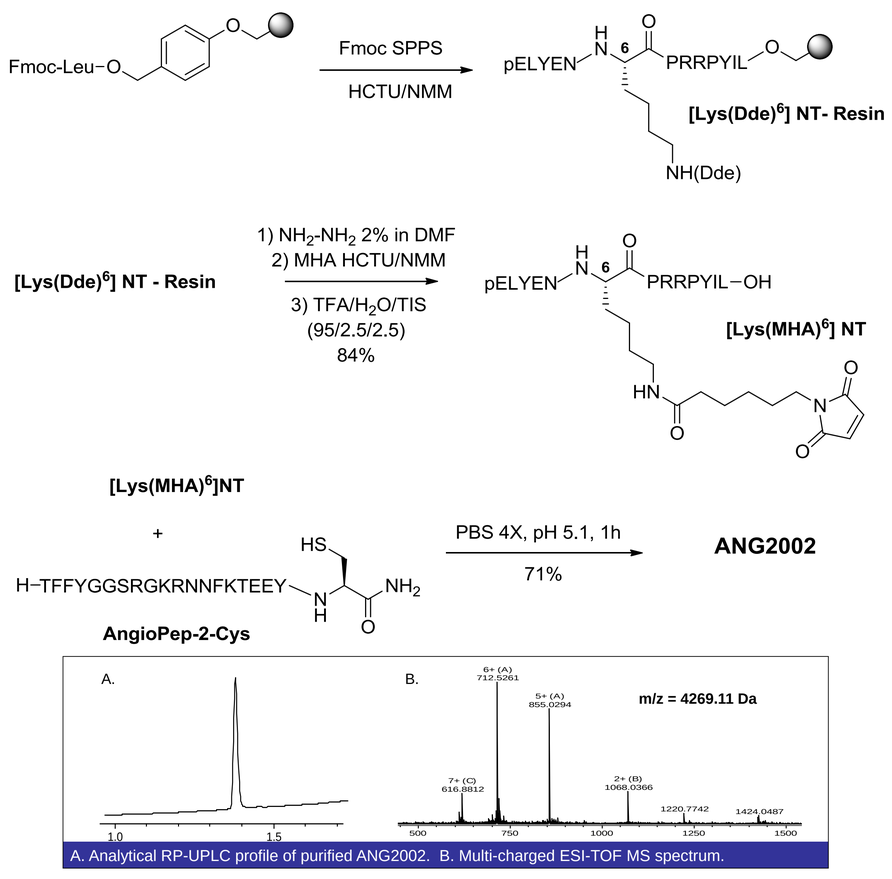
Washout with saline: 30s
Quantification of radioactivity in the brain

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 sub-plantar injection (0.02 ml, 2% solution).

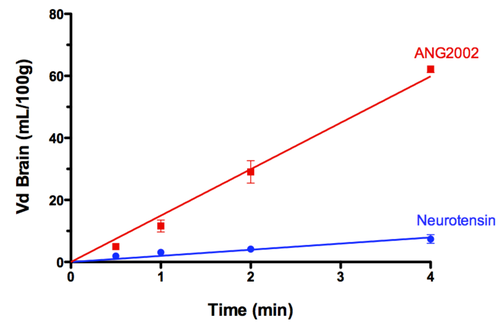
Synthesis of ANG2002



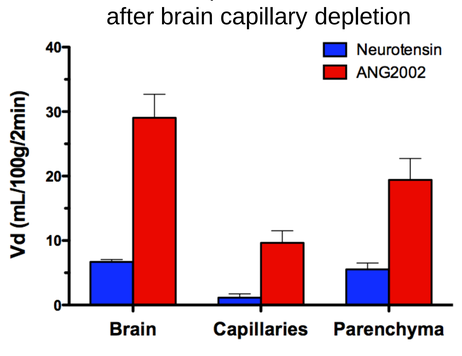
A. Analytical RP-UPLC profile of purified ANG2002. B. Multi-charged ESI-TOF MS spectrum.

Brain Uptake of ANG2002 and Binding Affinity to NT receptors

A. *In situ* brain perfusion

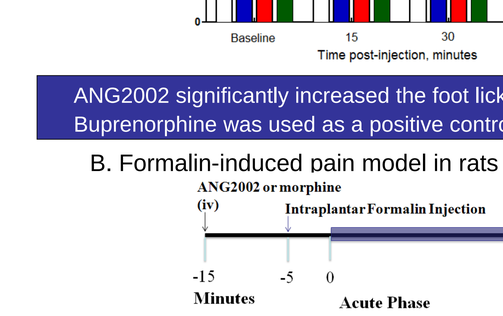


B. Brain compartment distribution after brain capillary depletion

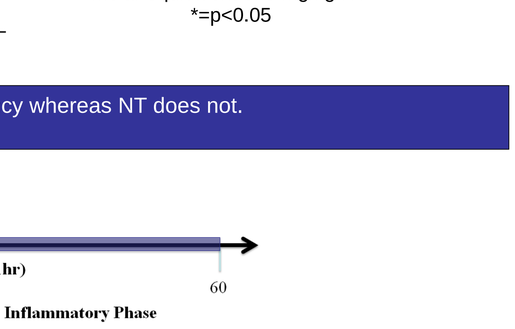


A. In vivo brain uptake of [^{125}I]-ANG2002 and [^{125}I]-Neurotensin measured by *in situ* brain perfusion.
B. Brain capillary depletion performed to assess the ANG2002 distribution in the brain compartments.

A. Binding to NTS1



B. Binding to NTS2

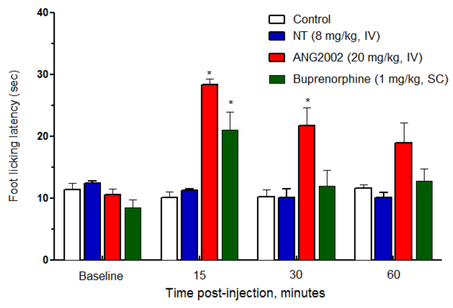


Compound	h NTS1 (IC_{50} nM)	h NTS2 (IC_{50} nM)
NT	4.0	4.0
ANG2002	13.0	3.3
ANG-NT(8-13)	NA	1.8

^{125}I Neurotensin competition binding on NTS1 (A) and NTS2 (B) receptors

Analgesic Properties of ANG2002

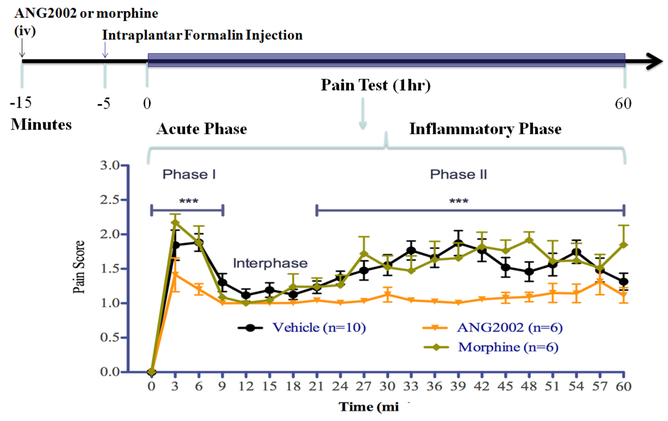
A. Hot plate assay in mice

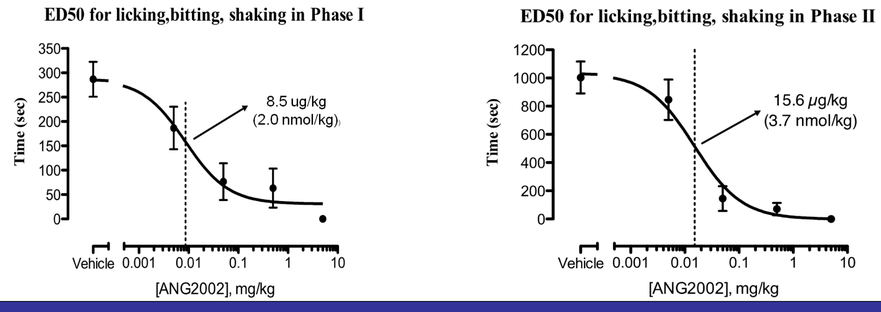


Note: 20 mg/kg ANG2002 is the molar equivalent of 8 mg/kg NT.
*=p<0.05

ANG2002 significantly increased the foot licking latency whereas NT does not. Buprenorphine was used as a positive control.

B. Formalin-induced pain model in rats





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

CONCLUSIONS

- **A new, high affinity, brain-penetrant NT agonist peptide-drug conjugate (ANG2002) is described.**
- **ANG2002 : Has a better brain penetration than native NT (10-folds higher)**
- **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 demonstrates the potential of Angiopep platform for the development of other neurotherapeutics with increased brain penetration.**