A new brain-penetrant Angiopep-2-morphine-6-glucuronide derivative (ANG2010) with analgesic properties

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ABSTRACT

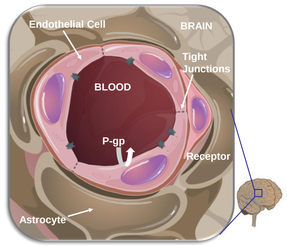
The blood-brain barrier (BBB), with tight junctions connecting brain capillary endothelial cells and high expression of active efflux transport proteins, has impeded development of new CNS therapeutics. The BBB serves as the natural gatekeeper of the brain, restricting entry of most pharmaceuticals while allowing essential molecules, such as glucose, insulin, and growth hormones to penetrate. Overcoming the obstacles posed by the BBB is a critical challenge for central nervous system (CNS) drug development. A new family of peptides derived from proteins that efficiently cross the BBB using low-density lipoprotein receptor related protein (LRP-1) 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 larger 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.

In the present study, we applied EPiC technology to the natural metabolite of morphine, morphine-6-glucuronide (M6G). The resulting new chemical entity, Angiopep-2-M6G (ANG2010), was evaluated for brain uptake and efficacy in models of analgesia. Despite the fact that M6G and morphine are almost equally potent after systemic administration, the analgesic potency of M6G has been shown to be 100-fold higher than morphine after intracerebral injection. However, the brain penetration of M6G is significantly lower than morphine, thus limiting its utility in pain management. Using an *in vivo* mouse paradigm, we observe a higher rate of brain penetration for the new chemical entity ANG2010 compared with that of unconjugated M6G and morphine. This increase in brain uptake results in a significant improvement in the pharmacological efficacy of M6G in the mouse hot plate and rat tail-flick assays. ANG2010 administration (i.v. or s.c.) induced a more prolonged duration of analgesia when compared with either M6G or morphine.

To evaluate the potential for GI side effects common to opiates, we determined gastrointestinal (GI) tract motility using the charcoal meal test in rats. While M6G and morphine significantly reduced GI transit time, the effect of ANG2010 after s.c. administration was less pronounced. In summary, we have introduced a new Angiopep-M6G derivative with improved BBB permeability, leading to potent analgesia and improving the GI side effect profile. Our data with ANG2010 further validates the use of EPiC technology for novel CNS treatments including pain management.

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.



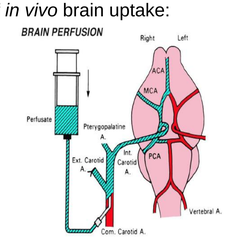
The BBB is a selective barrier formed by tightly packed endothelial cells that line the cerebral capillaries. The BBB is important as it provides an insulated environment for stable neuronal function. Endothelial cells forming the BBB are not only able to form tight junctions, but also possess the following 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. Morphine 6-glucuronide (M6G), one of the major metabolite of morphine, has been reported to be more potent than morphine to induce antinociception when directly injected into the brain. However, the poor penetration of M6G across the blood-brain barrier (BBB) limits its utilization as a therapeutic agent. In the present study, we investigated the brain uptake and analgesic effects of a new chemical entity formed by conjugation of M6G to Angiopep-2, a 19-mer peptide that crosses the BBB. Results of *in-situ* brain perfusions in mice demonstrated that the Angiopep-2-M6G conjugate, ANG2010, efficiently penetrated the blood-brain barrier with a transport rate at least 40-fold higher than that of unconjugated morphine or M6G. Importantly, ANG2010 exhibited activity in two animal pain models: 1) hot plate mouse model, and 2) rat tail flick pain model. In both models, ANG2010 induced a potent and more prolonged analgesia than morphine and M6G. At a more general level, the generation of ANG2010 further demonstrates the potential of the EPiC platform for the development of pain compounds with enhanced brain penetration.

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-ANG2010
^{3}H-morphine
Washout with saline: 30s
Quantification of radioactivity in the brain

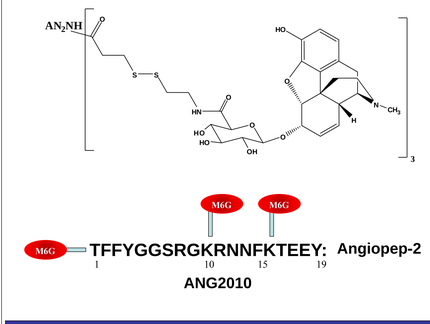
2. Evaluation of analgesic effect in pain models:

- **Hot plate mouse model**: Mice were placed onto a hot metal plate maintained at 54ºC and paw flick 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.

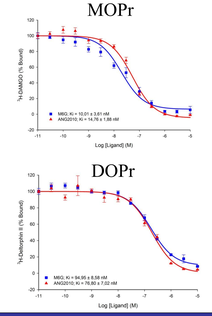
- **Rat tail flick model**: Pain threshold was measured before (baseline) and after drug administration, using a standard hot-water tail-flick assay. The dependent variable was the latency (in seconds) for the rat to flick its tail from the hot-water bath. The water was maintained at 53ºC in a constant-temperature water bath . The distal first 5 cm of the rat's tail was immersed in the bath, and the time required for the rat to remove its tail was measured. A statistically significant increase from baseline pain-threshold measurement was interpreted as induction of analgesia.

NEW M6G (ANG2010) DERIVATIVE

A. ANG2010 chemical structure



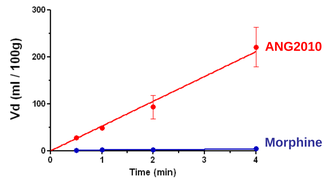
B. Competition binding assay on opioid receptors



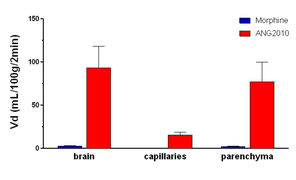
A.Chemical structure and schematic representation of the Angiopep-2-M6G conjugate (ANG2010). B. Competition binding of M6G and ANG2010 to Mu-opioid (MOPr) or Delta-opiod (DOPr) receptors in rat brain membranes. Brain membranes were incubated with either 1 nM [^{3}H]-DAMGO or 1 nM [^{3}H]-Deltorphin II and increasing concentrations (10^{-11} to 10^{-5} M) of unlabeled M6G or unlabeled ANG2010 for 60 minutes at 37 °C. Binding inhibition results were analyzed using GraphPad Prism software. Each curve represents the mean of 6 measurements. Data are expressed as the mean K_i with SEM.

BRAIN UPTAKE OF ANG2010

A. *In situ* brain perfusion



B. Brain compartment distribution of ANG2010 after brain capillary depletion



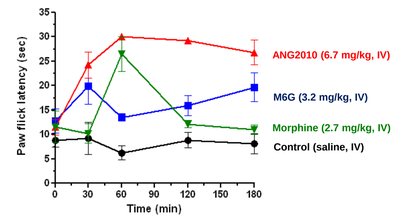
C. Initial brain transport rates (Kin)

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}
ANG2010 (Angiopep-2-Morphine 6-glucuronide)	**8.8 x 10^{-3}**
Alcohol	1.8 x 10^{-4}
Morphine	**2.2 x 10^{-4}**
Insulin Rec Antibody	1.0 x 10^{-4}
Paclitaxel and Doxorubicin	~5 x 10^{-5}
M6G	2.0 x 10^{-5}
mAb	~6 x 10^{-5}

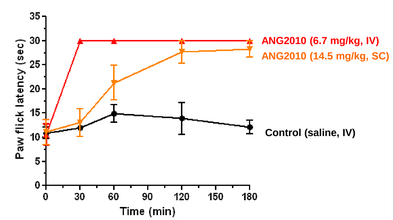
***In vivo* brain uptake of the [^{125}I]-ANG2010 and [^{3}H]-morphine was measured by *in situ* brain perfusion. B. Brain capillary depletion was performed to assess the ANG2010 distribution in the brain compartments. C. Initial brain transport rate (Kin) values for ANG2010, Morphine and M6G compared to that of other molecules.**

ANALGESIC PROPERTIES OF ANG2010

A. IV administration of ANG2010 and M6G (hot plate mouse assay)



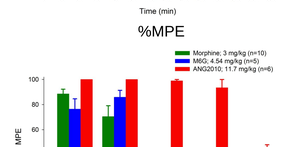
B. IV vs SC administration of ANG2010 (hot plate mouse assay)



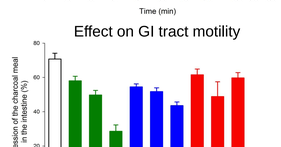
Effect of ANG2010 in the mouse hot plate assay. ANG2010 significantly increased the paw flick latency compared to unconjugated (M6G) after iv bolus injection at an equimolar dose.

ANG2010 after IV or SC administration significantly increases the paw flick latency for at least 3hrs.

A. Analgesic effect of i.v. morphine, M6G and ANG2010



A. Analgesic effect of s.c. morphine, M6G and ANG2010



B. %MPE

B. Effect on GI tract motility

(A) Analgesic effect of ANG2010 compared to that of unconjugated morphine and M6G was evaluated in the rat tail flick model after IV bolus injection (equivalent to 3 mg/kg of morphine sulfate). (B) Results were represented as the Maximal Possible Effect (%) for the three drugs.

(A) Analgesic effect of ANG2010 compared to that of unconjugated morphine and M6G was evaluated in the rat tail flick model after SC Bolus injection (equivalent to 3 mg/kg of morphine sulfate) (n = 10 rats/group). (B) Effect of SC ANG2010 on the gastro-instestinal transit (n = 10 rats/group).

CONCLUSIONS

- In addition to the anticancer agent, GRN1005, which shows promising results in Phase 1/2 studies for brain tumors, new Angiopep-2-M6G (ANG2010) has been generated for pain.
- In the present study, the main objective was to broaden the EPiC platform for morphine derivatives by generating a new Angiopep-2-M6G derivative (ANG2010) for pain.
- ANG2010:
  - Crosses the BBB more efficiently than native morphine and M6G
  - Binds to opioid receptors with affinity similar to that of native M6G
  - Demonstrates *in vivo* efficacy (iv or sc) in two rodent pain models: Hot plate mouse model and rat tail flick model
- Strong validation of the EPiC platform for small molecules opens new avenues for other potential pain compounds that do not cross the BBB.

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