655.15 / DD18 : Angiopeps: a new peptide family for the transport of small and large molecules to the brain

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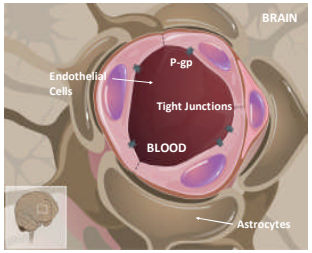
ABSTRACT

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, including P-glycoprotein (Pgp). As a result, the overwhelming majority of small molecules, proteins and peptides do not cross the BBB. In the present study we provide experimental evidence that a new peptide-based drug delivery technology platform (Angiopep™) provides a non-invasive and flexible platform for transporting drugs into the central nervous system. The platform consists of a family of peptides derived from a naturally-occurring protein capable of crossing the blood-brain barrier (BBB). Lead carrier peptides were evaluated in an in vitro model of the BBB and in vivo by in-situ brain perfusion and a non-invasive optical imaging in mice using radioactively-labeled or peptides conjugated with the near-infrared probe Cy5.5, respectively. Thanks to the *in-vitro* model of the BBB we were able to show that angiopep-2 transcytosis was mediated in part by a receptor, the Low density lipoprotein receptor Related Protein (LRP). Fluorescence associated to angiopep-2-cy5.5 was detected very rapidly in the brain parenchyma after in-situ brain perfusion in mice and higher fluorescence was measured in the brain tumor tissue compared to normal brain parenchyma. Based on these properties, we have created several new drug entities, the most advanced of which is ANG1005 formed by chemical conjugation of our peptide vector (angiopep-2) to three molecules of paclitaxel. Using in-situ brain perfusion in rats we were able to demonstrate that the transport rate across the BBB of ANG1005 is approx. 100 x higher than that of paclitaxel. Furthermore ANG1005 is homogenously distributed in the brain. High concentration of ANG1005 in the brain parenchyma (700 nM) is measured by LC-MS-MS which corresponds to 2.1 µM of paclitaxel well above therapeutic levels. In a rat orthotopic glioblastoma (U87) tumor model, administration of ANG1005 resulted in a shrinking of IC tumors as measured by MRI confirming that this vector platform technology can deliver a therapeutic amount of paclitaxel to the brain tumors. ANG1005 is currently under evaluation in two phase 1/2 clinical trials for the treatment of primary and secondary brain tumors in humans.
By using our platform technology and creating new drug by chemical conjugation to the AngioPep family of peptides we allow access to the brain parenchyma of small drugs and larger hydrophilic drugs such as antibodies. All new drugs created with our technology are patentable NCEs.

INTRODUCTION

The BBB is a unique, 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).



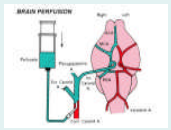
Existing drug candidates (mostly biologics) available to address conditions localized in the brain have limited to no therapeutic value in-vivo due to the fact that they do not cross the BBB to reach the site of disease.

To overcome this obstacle, Angiochem has developed a new vector technology based on chemically attaching small molecules, peptides, monoclonal antibodies, siRNA, etc… to a vector that shuttles them into the brain using LRP receptors that are naturally expressed at the BBB.

EXPERIMENTAL MODELS

1. **Brain tumor distribution after IV injection of fluorescent conjugates (Angiopep-2-cy5.5 and Angiopep-7-cy5.5) in mice :**
   - Mice were intracranially implanted with 70,000 U87 (human glioblastoma) cells
   - Animals were intravenously injected with fluorescent conjugates 10 days after implantation
   - Injected animals were viewed 24 hours after injection in the near-infrared mode (Red) using 660-680 nm excitation and 700 nm longpass emission filter under a Zeiss Axiovert 200 fluorescent microscope developed by Carl Zeiss.
2. **Brain parenchyma distribution of fluorescent conjugate (Angiopep2-cy5.5 and Angiopep7-cy5.5) after in-vivo injection in mice:**
   - Mice were IV injected in the tail vein with 100 µg of fluorescent Angiopep-2 and Angiopep-7
   - After 24 hrs, the brain was perfusion with physiological saline alone and fixed with formalin/saline solution
   - Vibrotome brain sections (50 µm thickness) were obtained and were viewed in the near-infrared mode (Red) (a 660- to 680-nm excitation and a 700-nm longpass emission filter) using Zeiss Axiovert 200 fluorescent microscope (Carl Zeiss).
3. **Normal brain uptake of ANG1005 after IV injection in mice:**
   - Animals were intravenously injected with ANG1005
   - Brain tissue was extracted 15 minutes after injection
   - Tissue levels of ANG1005 were measured by HPLC (data was confirmed by LC-MS-MS)
4. **Efficacy of ANG1005 compared to paclitaxel and vehicle in a rat tumor model*:**
   - Animals were intracranially implanted with U87 cells
   - Animals were intraperitoneally injected with ANG1005, paclitaxel, or vehicle twice weekly starting 10 days after implantation
   - Efficacy was evaluated by following tumor size by MRI

   *Study performed by Oncodesign Technologies
5. **K_{in} (BBB transfer constant) *and regional distribution* of radioactive ANG1005 and other conjugates using *in situ* brain perfusion in rats and in mice**
   - Animals were perfused with physiological saline and radioactive ANG1005 or other conjugates at a rate of 5 mL/min in rats and 1.15 ml in mice for periods of between 15 seconds and 15 minutes
   - Animals were sacrificed immediately thereafter allowing brain dissection and subsequent regional distribution assessment



ANGIOPEP-2 VECTOR

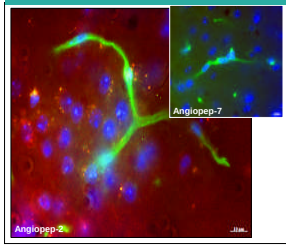
Angiopep-2: TFFYGGSRGKRNNFKTEEY

VECTOR CHOSEN FOR DEVELOPMENT

Angiopep-7: TFFYGGSRGRRNNFRTEEY

NEGATIVE CONTROL

ANGIOPEP2-CY5.5 IN THE BRAIN PARENCHYMA

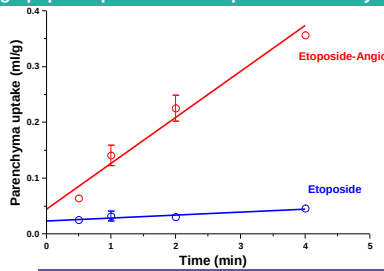


- ANGIOPEP LABELED WITH CY5.5
- CAPILLARIES STAINED WITH VESSEL GREEN (FITC-LECTIN)
- NUCLEI OF BRAIN CELLS STAINED WITH DAPI BLUE

Angiopep-2-cy5.5 is localized in the brain parenchyma 24 hr after iv administration (tail vein) in mice, contrarily to Angiopep-7 which does not cross the BBB

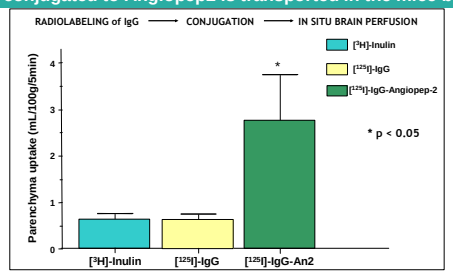
ANGIOPEP, a new vector for the transport of large or small therapeutic compounds

Angiopep2-etoposide is transported efficiently in mice brain



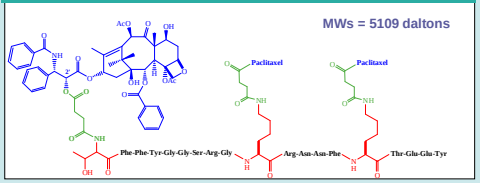
Etoposide-Angiopep2: K_{in} = 1.4 x 10^{-3} (ml/g/s)
Etoposide : K_{in} = 9.0 x 10^{-5} (ml/g/s)

IgG conjugated to Angiopep2 is transported in the mice brain



ANGIOPEP-2 PACLITAXEL CONJUGATE AS A PROOF OF CONCEPT

ANG1005 (Angiopep-2-3 Paclitaxel)



ANG1005 allows Therapeutical Concentration of Paclitaxel to be delivered to the brain

Mouse brains were analysed by HPLC post ANG1005 bolus injection (30mg/kg IV)
- ANG1005 quantity: 3.92 µg/g
- Concentration: 700 nM (2,100 nM of paclitaxel equiv.)

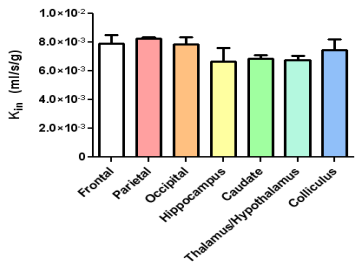
ANG1005 allows Delivery of 100 times the concentration of Paclitaxel required for Activity (20 nM)

Transport Rate of ANG1005 in the Rat Brain

DRUG	BRAIN K_{in} (ml/s/g)
ANG1005	8.800 ± 0.6 x 10^{-3}
Temozolomide	1 ± 0.1 x 10^{-3}
Angiopep-2	8.8 ± 0.13 x 10^{-4}
Paclitaxel	8.5 ± 5 x 10^{-5}
Etoposide	~4 x 10^{-6}

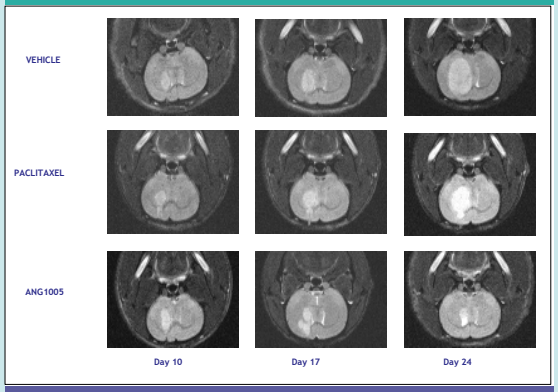
Initial transport rate measured by in-situ brain perfusion in rats demonstrate that ANG1005 is 10 x and 100x better transported than Angiopep-2 and Paclitaxel, respectively

Homogenous Distribution of ANG1005 In The Brain



ANG1005 is measured after 5 min in situ brain perfusion in rats. Data show homogenous distributed in normal rat brain of ANG1005

ANG1005 Concentration is effective for the treatment of Brain tumor in Rats



ANG1005 Treatment results in tumor regression. This was not the case with rats treated with paclitaxel or vehicle alone, which results in the growth of the U87 implanted tumor as measured by MRI. In addition, no brain tumor were detected in 5 out of 8 rats treated with ANG1005.

CONCLUSIONS :

- Angiopep-2 is rapidly transported to the brain parenchyma IV injection in mice
- 100 times more ANG1005 is transported into brain parenchyma as compared to Paclitaxel (in-situ rats)
- Therapeutical amounts of Paclitaxel is delivered in the brain using ANG1005.
- Inhibits intracranial tumor growth as measured by MRI in rats
- Angiopep-2 can transport other small drugs (Etoposides) and large protein (monoclonal antibodies) across the BBB.
- Angiopep-2 is a new platform technology for the delivery of therapeutical compounds to the CNS for the treatment of brain diseases.

Ref.: Demeule et al., JPET 324:1064-1072, 2008
Demeule et al., J. Neurochem 106:1534-1544, 2008
Regina et al., Br J Pharmacol 155:185-197, 2008