Poster 117 : ANG1005, Paclitaxel conjugated to the Angiopep brain transport vector for the treatment of brain cancer: preclinical studies

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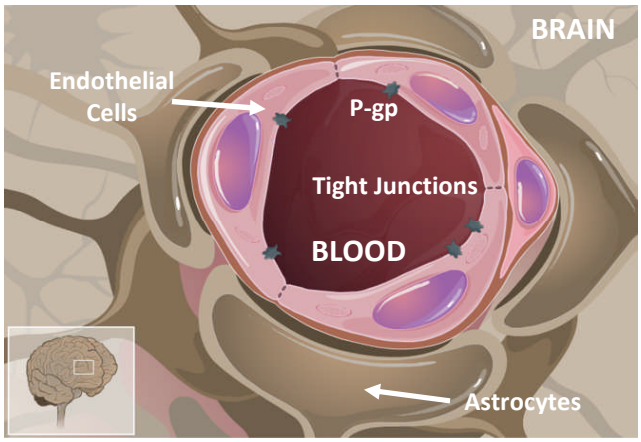
ABSTRACT

Background: 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 investigated the utilization of a new peptide based drug delivery technology that provides a non-invasive and flexible platform for transporting drugs into the central nervous system. **Material and Methods:** *In situ* brain perfusion were used to assess the brain uptake of our conjugates. Analysis of tissues was done after extraction by LC-MS-MS or HPLC. Xenograft models of glioblastoma (U87) were established by intracranial stereotaxic injections of U87 cells in mice and rats. **Results:** Angiopep2-Cy5.5 is very rapidly transported in the brain parenchyma as visualized in the brain after intravenous and in-situ brain perfusion. Higher fluorescence was also detected in the brain tumor compared to the normal brain. 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. In contrast to free paclitaxel, which is normally prevented from reaching the brain by the Pgp efflux pump, ANG1005 is efficiently transported across the BBB, with approx. 100 fold higher transport rate compared to free paclitaxel and 10 fold higher transport rate than Temozolomide measured using in-situ brain perfusion in rats. In addition, ANG1005 is homogenously distributed in rat brains. ANG1005 was detected by LC-MS-MS in both normal brain and brain tumors in mice 30 minutes after i.v. injection; detected brain levels of 2.1 µM are above the therapeutic concentrations of paclitaxel. The effect of ANG1005 was evaluated on glioblastoma (U87) xenograft tumor growth in immune deficient mice and resulted in a significant increase of survival of mice treated with ANG1005 of 27%. In a rat glioblastoma (U87) brain orthotopic model, administration of ANG1005 resulted in a shrinking of IC tumors measured by MRI. **Conclusion:** The Angiopep peptide vector can be used to transport small drugs to the brain parenchyma for the treatment of brain cancers. ANG1005 is currently under evaluation in two phase 1 clinical trials for the treatment of primary and secondary brain tumors in humans.

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).



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 (Angiopep-2-cy5.5) after in-situ mice brain perfusion :**
   - Mice were perfused in the carotid artery with physiological saline and conjugate (2 µM) at a rate of 1.15 ml per min for 10 min
   - After 10 min, the brain was further 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

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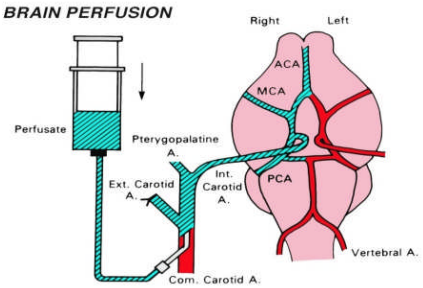
- Tissue levels of ANG1005 were measured by HPLC (confirmed by LC-MS-MS)

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. ***Kin* (BBB transfer constant) *and regional distribution* of radioactive ANG1005 using *in situ* brain perfusion in rats**
   - Animals were perfused with physiological saline and radioactive ANG1005 at a rate of 5 mL/min for periods of between 15 seconds and 15 minutes
   - Animals were sacrificed immediately thereafter allowing brain dissection and subsequent regional distribution assessment.

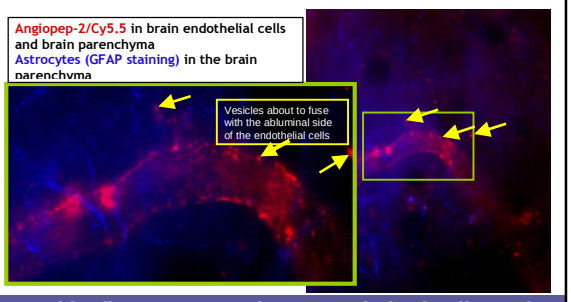


ANGIOPEP-2 VECTOR

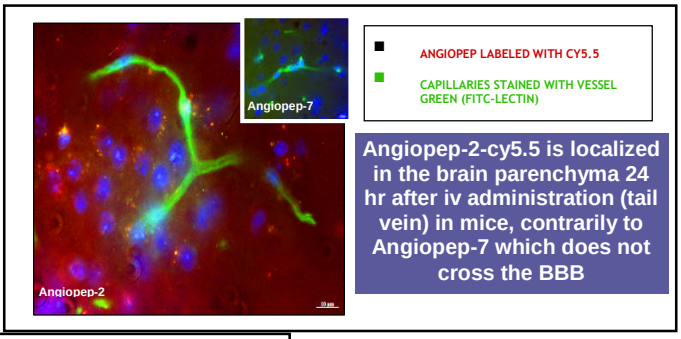
Angiopep-2: TFFYGGSRGKRNNFKTEEY
VECTOR CHOSEN FOR DEVELOPMENT

Angiopep-7: TFFYGGSRGRRNNFRTEEY
NEGATIVE CONTROL

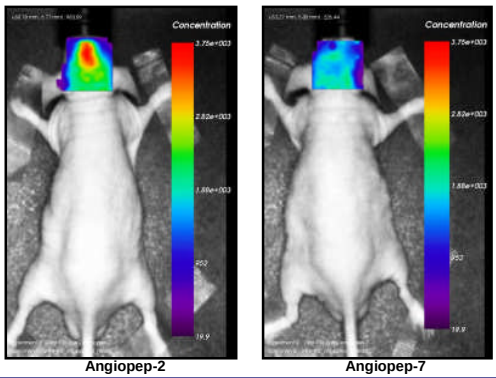
ANGIOPEP2-CY5.5 IN THE BRAIN PARENCHYMA



Detected by fluorescence microscopy in brain slices after 10 min in-situ brain perfusion in mice

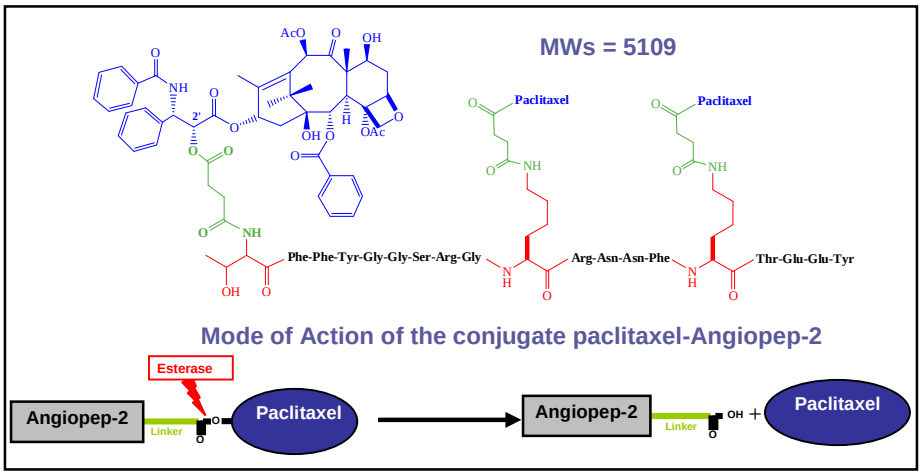


Angiopep-2-cy5.5 is localized in the brain parenchyma 24 hr after iv administration (tail vein) in mice, contrarily to Angiopep-7 which does not cross the BBB



High Distribution of Angiopep2-cy5.5 in the brain tumor area as compared to the negative control measured by in-vivo fluorescence imaging in mice IC implanted with U87 glioblastoma cells

ANG1005 AS A PROOF OF CONCEPT
Conjugate between Angiopep-2 and Paclitaxel

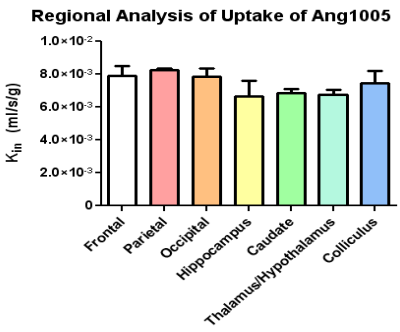


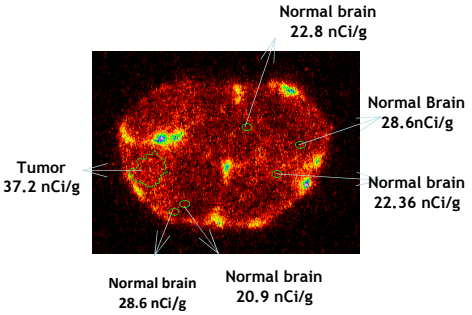
TRANSPORT RATE OF ANG1005 TO THE BRAIN

DRUG	BRAIN K_{in} (ml/s/g x 10^{-6})
ANG1005	8800 ± 600
Temozolomide	1000 ± 100
Angiopep-2	880 ± 130
Paclitaxel	85 ± 5
Doxorubicin	~50
Gemcitabine	13 ± 1.4
Etoposide	~4

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 PARENCHYMA





ANG1005 is measured after 5 min in situ brain perfusion in rats and by brain autoradiography with brain slices measured 30 min after IV injection of radiolabeled ANG1005 in mice. Data show in both rats and mice that ANG1005 is homogenously distributed in normal brain

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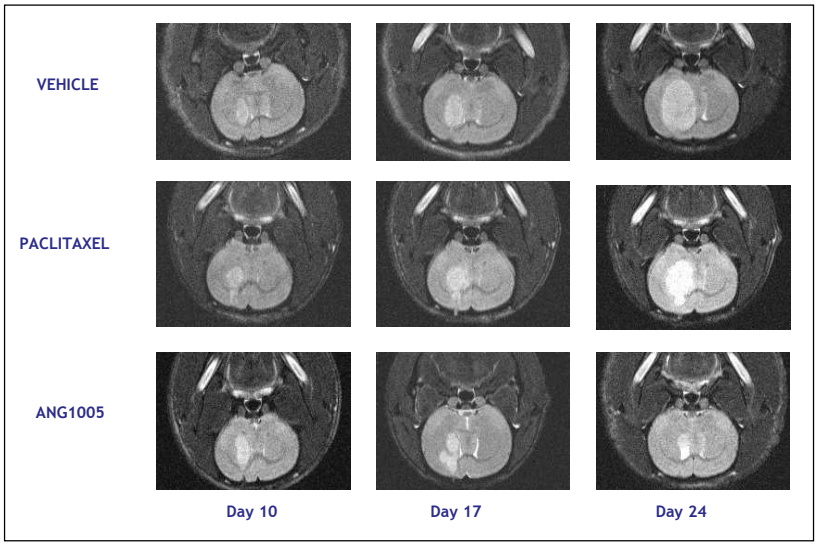
ANG1005 ALLOWS THERAPEUTICAL CONCENTRATION OF PACLITAXEL TO BE DELIVERED TO THE BRAIN

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)

ANG1005 AS A PROOF OF CONCEPT
Conjugate between Angiopep-2 and Paclitaxel



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**
- **Angiopep2 shows higher distribution in brain tumors**
- **100 times more ANG1005 is transported into brain parenchyma as compared to Paclitaxel**
- **Homogenous distribution of ANG1005 in brain regions**
- **Therapeutical amounts of Paclitaxel is delivered in the brain using ANG1005.**
- **Inhibits intracranial tumor growth as measured by MRI in rats**

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
Posters # 139, 424 and 425

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