ET-09 : A new drug, ANG1005, a conjugate of Paclitaxel and Angiopep peptide vector able to cross the Blood-Brain Barrier for the treatment of brain cancers.

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ABSTRACT

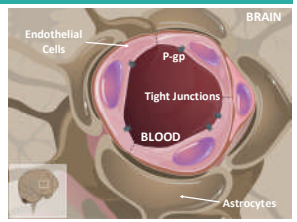
The blood-brain barrier (BBB) is mainly formed by brain capillary endothelial cells which are closely sealed by tight junctions. This important characteristic provides a natural defense against toxic or infective agents circulating in the blood. Furthermore, brain endothelial cells possess few alternative transport pathways 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. Therefore, the development of a drug delivery system for the brain is of great interest for the treatments of neurological disorders. 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 BBB. The Angiopeps cross the BBB using a receptor mediated mechanism involving the Low density lipoprotein receptor Related Protein (LRP). The lead carrier peptide (Angiopep-2) was evaluated in vivo by in-situ brain perfusion and by non-invasive optical imaging in mice using radioactively-labeled or peptides conjugated with the near-infrared probe Cy5.5, respectively. Angiopep-2 peptides were detected very rapidly in the brain parenchyma. Higher fluorescence associated to Angiopep-2-cy5.5 was detected in the brain tumor compared to the normal brain. Based on this discovery, 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 BBB P-glycoprotein (P-gp) efflux pump, ANG1005 is efficiently transported across the BBB, with approx. 100 fold higher transport rate compared to free paclitaxel and 10 fold higher compared to temozolomide (TMZ). Furthermore, 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; brain levels of 700 nM correspond to 2.1 µM which is above the therapeutic concentrations of paclitaxel. Based upon the higher distribution of ANG1005 in brain tumors, 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. Using this platform technology we can transport small anti-cancer drugs and larger molecules across the BBB. ANG1005 is currently under evaluation in two phase 1 clinical trials for the treatment of primary and metastatic 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).

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.

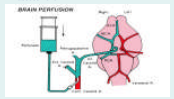


To overcome this obstacle, Angiochem has developed a new vector technology based on chemically attaching small molecules, peptides, monoclonal antibodies, siRNA, etc… to a vector that shuttles them into the brain using LRP receptors that are naturally expressed at the BBB.

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 the 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 perfused with physiological saline alone and fixed with formalin/saline solution
   - Vibratome 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 (exp 1) injected or administered by IV infusion (exp 2) 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 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

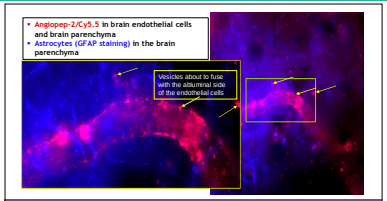
Angiopep-2: TFFYGGSRGKRNNFKTEEY

VECTOR CHOSEN FOR DEVELOPMENT

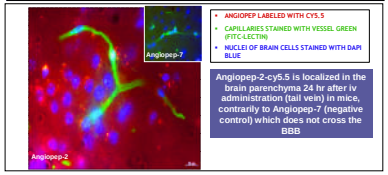
Angiopep-7: TFFYGGSRGRRNNFRTEEY

NEGATIVE CONTROL

ANGIOPEP2-CY5.5 IN THE BRAIN PARENCHYMA

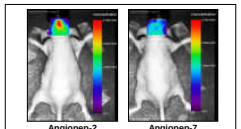


Detected by fluorescence microscopy in brain slices after 10 min in-situ brain perfusion in mice

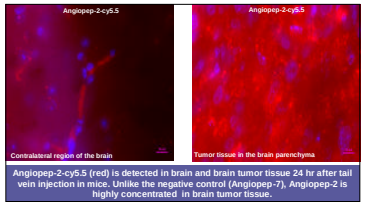


Angiopep-2-cy5.5 is localized in the brain parenchyma 24 hr after iv administration (tail vein) in mice, contrarily to Angiopep-7 (negative control) which does not cross the BBB

ANGIOPEP2-CY5.5 IN THE BRAIN PARENCHYMA AND IN BRAIN TUMOR

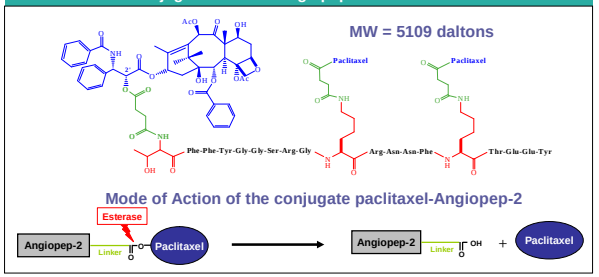


High Distribution of Angiopep-2-cy5.5 in the brain tumor area as compared to the negative control



Angiopep-2-cy5.5 (red) is detected in brain and brain tumor tissue 24 hr after tail vein injection in mice. Unlike the negative control (Angiopep-7), Angiopep-2 is highly concentrated in brain tumor tissue.

ANG1005 as Proof of Concept: Conjugate between Angiopep-2 and Paclitaxel

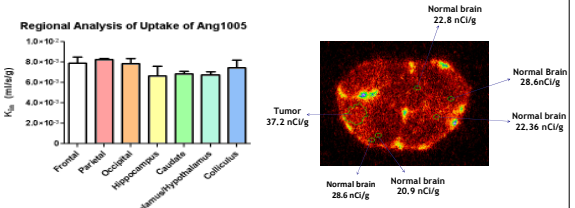


Transport Rate of ANG1005 to the Brain

DRUG	BRAIN K_{in} (ml/s/g)
ANG1005	$8.8 \pm 0.6 \times 10^{-3}$
Temozolomide	$1 \pm 0.1 \times 10^{-3}$
Angiopep-2	$8.8 \pm 1.3 \times 10^{-4}$
Paclitaxel	$8.5 \pm 0.5 \times 10^{-5}$
Doxorubicin	$\sim 5 \times 10^{-5}$
Gemcitabine	$1.3 \pm 0.14 \times 10^{-5}$
Etoposide	$\sim 4 \times 10^{-6}$

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

Homogenous Distribution of ANG1005 in Brain Parenchyma



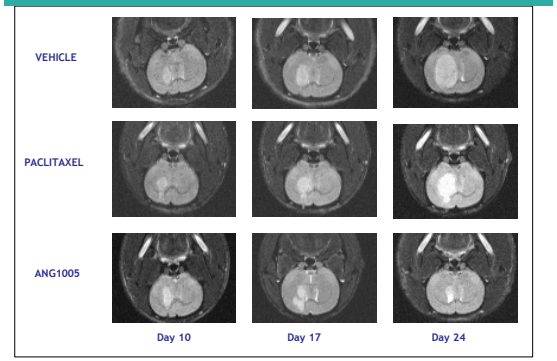
ANG1005 is measured 5 min post in-situ brain perfusion in rats and by brain autoradiography with brain slices measured 30 min after IV injection of radiolabeled ANG1005 in mice. Rat and mice data both show that ANG1005 is homogenously distributed in normal brain.

ANG1005 allows Therapeutic Concentrations of Paclitaxel to be Delivered to the Brain

Mouse brains were analysed by HPLC post ANG1005 bolus injection
- 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)

Experiment 1: ANG1005 Concentration is Effective in Treating Brain Tumors in Rats after IP Administration



Experiment 2	Mean tumor volume (mm^3)	
Treatment	D10	D21
Vehicle	9 ± 4 (n=4)	25 ± 20 (n=4)
ANG1005, IV infusion, 6 mg/kg/inf	4 ± 3 (n=4)	8 ± 5 (n=2)
Taxol® IV bolus, 5 mg/kg/inj	6 ± 2 (n=4)	19 ± 2 (n=4)

ANG1005 treatment resulted in tumor regression in 2 separate experiments. These experiments demonstrate that a therapeutic concentration of paclitaxel is transported across the BBB in rats after IP (1) and IV (2) administration.

CONCLUSIONS :

- Angiopep-2 is rapidly transported to brain parenchyma
- Angiopep-2 shows higher distribution in brain tumors
- ANG1005 transport into brain parenchyma is 100 times higher than paclitaxel
- ANG1005 distributes homogenously in brain regions
- ANG1005 delivers therapeutic concentrations of paclitaxel to the brain
- 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