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.

Reinhard Gabathuler[1], Michel Demeule[1], Anthony Régina[1], Christian Ché[1], Paul Lockman[3], Fancy Thomas[3], Julie Gaasch[3], Helen Thorsheim[3], Abedelnasser Abulrob[4], Quentin R. Smith[3], Danica Stanimirovic[4], Richard Béliveau[2] and Jean-Paul Castaigne[1]

[1]Angiochem Inc., Montréal, QC, Canada, [2]Université du Québec à Montréal, Montréal, QC, Canada, [3]Texas Tech University HSC, Amarillo, TX and [4] NRC Institute for Biological Sciences, Ottawa, ON, Canada

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 new technology to cross the BBB for brain parenchyma uptake is of great interest for the treatments of neurological disorders. Angiochem's proprietary EPiC platform targets the low-density lipoprotein receptor-related protein (LRP) receptor family. In the present studies, we provide experimental evidence that these engineered peptide compounds (EPiC) are new drugs developed for the treatment of diseases of the central nervous system. The new compounds composed of a peptide backbone cross the BBB using a receptor mediated mechanism involving the Low-density lipoprotein Receptor-related Protein (LRP). The lead 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 which is a new antimicrotubule agent that contains the proprietary sequence of amino acids responsible for receptor-mediated transcytosis across the BBB. 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 <<remove this: 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.

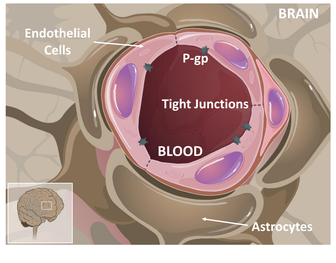
INTRODUCTION

Angiochem Inc. is a clinical-stage biotechnology discovering and developing new breakthrough drugs that are uniquely capable of crossing the BBB to treat brain diseases These new Engineered Peptide Compounds (EPiC) have the potential to address significant medical needs many of which cannot be effectively addressed due to the fundamental physiological challenge presented by the BBB.

The BBB is a unique, selective barrier formed by tightly packed endothelial cells that line the cerebral capillaries. 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.

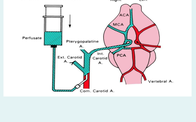


Angiochem has developed new Engineered Peptide Compounds (EPiC) and synthesized new drugs based on a variety of active compounds, small molecules, peptides, monoclonal antibodies, siRNA, etc… to treat brain diseases using naturally expressed LRP receptors at the BBB.

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
   - 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 (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

Angiopep-2: TFFYGGSRGKRNNFKTEEY

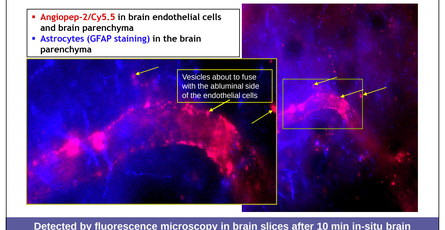
PEPTIDE CHOSEN FOR DEVELOPMENT

Angiopep-7: TFFYGGSRGRRNNFRTEEY

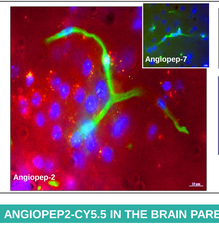
NEGATIVE CONTROL

ANGIOPEP2-CY5.5 IN THE BRAIN PARENCHYMA

- Angiopep-2/Cy5.5 in brain endothelial cells and brain parenchyma
- Astrocytes (GFAP staining) in the brain parenchyma



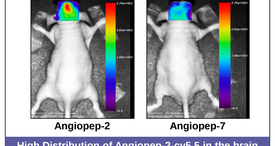
Detected by fluorescence microscopy in brain slices after 10 min in-situ brain perfusion in mice



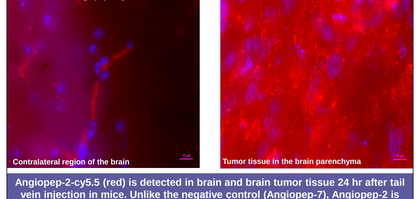
- ANGIOPEP LABELED WITH CY5.5
- CAPILLARIES STAINED WITH VESSEL GREEN (FITC-LECTIN)
- NUCLEI OF BRAIN CELLS STAINED WITH DAPI BLUE

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

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

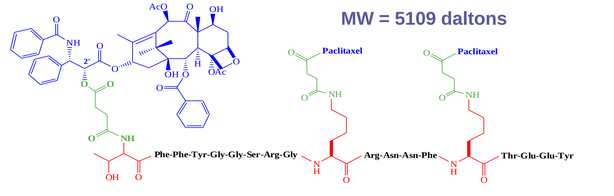


High Distribution of Angiopep-2-cy5.5 in the brain tumor area as compared to the negative control



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: New antimicrotubule agent using EPiC technology

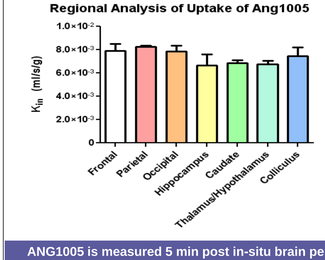


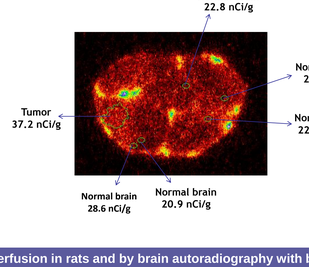
Transport Rate of ANG1005 to the Brain

DRUG	BRAIN K_{in} (mL/s/g)
ANG1005	8.8 0.6 x 10^{-3}
Temozolomide	1 0.1 x 10^{-3}
Angiopep-2	8.8 1.3 x 10^{-4}
Paclitaxel	8.5 0.5 x 10^{-5}
Doxorubicin	~5 x 10^{-5}
Gemcitabine	1.3 0.14 x 10^{-5}
Etoposide	~4 x 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





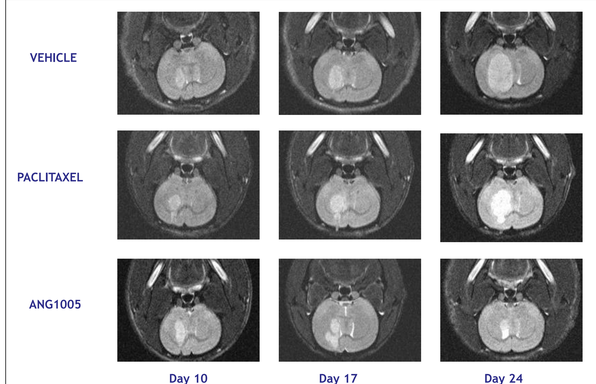
ANG1005 is measured 5 min post in-situ brain perfusion in rats and by brain autoradiography with brain slices measured 30 min after IV injection of radiolabeled ANG1005 in mice. Rat and mice data both show that ANG1005 is homogenously distributed in normal brain.

ANG1005 reaches Therapeutic Concentrations of Paclitaxel in 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