Abstract #3784:

Development of new engineered peptide compound (EPiC) platform for the transport of therapeutics to the brain

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ABSTRACT

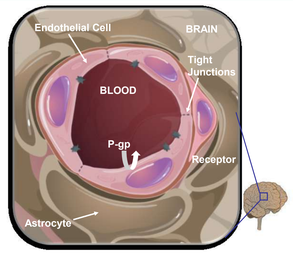
The blood-brain barrier (BBB) is mainly formed by brain capillary endothelial cells which are closely sealed by tight junctions and express high levels of active efflux transport proteins, including P-glycoprotein (Pgp). As a result, the overwhelming majority of small molecules, proteins and peptides do not cross the BBB. Angiochem's engineered peptide compounds (EPiC) provides a non-invasive and flexible platform for small and large molecules to treat brain diseases. Based on these properties, we have created a portfolio of new drug entities composed of siRNA, peptides and MAbs, the most advanced of which is ANG1005 formed by chemical conjugation of our peptide to three molecules of paclitaxel. ANG1005 has shown efficacy in animal models with brain tumors and is currently under evaluation in two phase ½ clinical trials for the treatment of primary and secondary brain tumors in humans. In the present study, we have investigated the brain uptake of new chemical entities formed by conjugation of the peptide Angiopep-2 (An2) with 3 molecules of the anti-cancer drugs doxorubicin or etoposide. Despite the clinical effectiveness of doxorubicin and etoposide in the treatment of many malignant tumors, clinical trials involving systemic administration have demonstrated very limited efficacy in the treatment of gliomas. This limited efficacy can be explained by the poor penetration of the drug thru the BBB and by the effect of the multidrug resistance pump, P-gp. Here, we show by mice *in-situ* brain perfusion that the etoposide and doxorubicin conjugates are transported very efficiently across the BBB. The transport rate is higher than that of unconjugated doxorubicin and etoposide by at least 5 to 10-fold. *In vitro*, both etoposide-An2 and doxorubicin-An2 conjugates inhibit cancer cell proliferation, with highly cytotoxic activities against various tumor cell lines. Both anticancer drug-An2 conjugates present comparable cytotoxicity to that of their unconjugated form. In addition, brain perfusion studies performed with P-gp knock-out mice showed that the conjugate bypasses the drug efflux pump P-gp at the BBB. In conclusion, these data confirm that conjugation of drug to the peptide Angiopep-2 significantly enhances their entry into the brain and further validate the use of Angiochem's technology for improving drug access to the brain.

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:

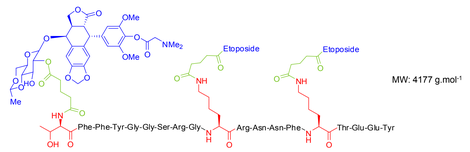
- ✓Lack fenestra;
- ✓Lack transendothelial channels;
- ✓Lack pinocytic vesicles; and
- ✓Express high levels of the active efflux pump (P-gp).



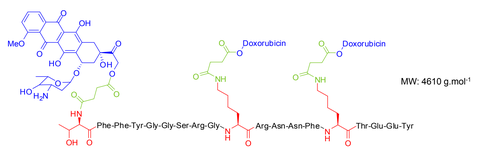
Angiochem's proprietary EPiC platform targets the low-density lipoprotein receptor-related protein (LRP) receptor family. This endogenous transcytosis system has a number of inherent biochemical advantages for drug transport across the BBB, including high expression, rapid turnover, numerous ligands of varying sizes, and limited down-regulation. LRP has been shown to be upregulated in primary brain tumors, brain metastases from lung cancers, a human hepatocellular carcinoma cell line, ovarian cancers, breast cancers, and melanomas (Bu *et al*., 1993; Moestrup *et al*., 1995; Grimsley *et al*., 1997; Orlando *et al*., 1997; Gliemann, 1998; Hussain, 2001). ANG1005 is an antimicrotubule agent that contains the proprietary sequence of amino acids responsible for receptor-mediated transcytosis across the BBB. Following intravenous administration, ANG1005 conjugate reaches the cancer cells in the brain and is internalized, with subsequent esterase cleavage to release paclitaxel. In the present study, two other anticancer drugs, etoposide and doxorubicin, were modified with Angiopep-2.

NEW ANTICANCER DRUG STRUCTURES

A. Etoposide-Angiopep-2



B. Doxorubicin-Angiopep-2



Two new anticancer drugs were generated by conjugating three molecules of etoposide (A) or doxorubicin (B) on one molecule of Angiopep-2.

RESULTS

ANGIOPEP-2

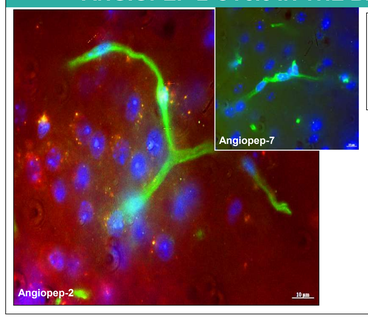
Angiopep-2: TFFYGGSRGKRNNFKTEEY

PEPTIDE CHOSEN FOR DEVELOPMENT

Angiopep-7: TFFYGGSRGRRNNFRTEEY

NEGATIVE CONTROL

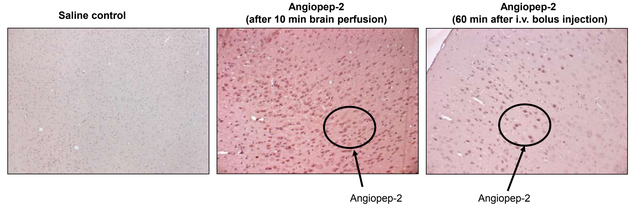
ANGIOPEP-2-CY5.5 IN THE BRAIN PARENCHYMA



- 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

IMMUNOHISTOCHEMISTRY OF ANGIOPEP-2 IN BRAIN



Angiopep-2 is detected in mice brain parenchyma using a rabbit anti-Angiopep-2 pAb after a 10 min *in situ* brain perfusion (4 µM) or 60 min after i.v. administration (20 mg/kg)

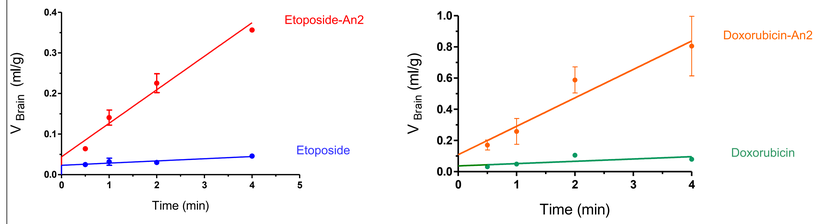
INHIBITION OF CANCER PROLIFERATION IN VITRO

Drugs	IC50 (nM) Glioblastoma (U87)	Hepatocarcinoma (SK-Hep-1)	Lung carcinoma (NCI-H460)
Etoposide	145	62	90
Etoposide-An2	330	48	148
Doxorubicin	18	10	11
Doxorubicin-An2	6.0	4.6	7.3

Effect of Etoposide-An2 and Doxorubicin-An2 on cancer cell proliferation. Cancer cells were incubated for 48 hrs with the new anticancer conjugates or with the unmodified drugs. [3H]Thymidine incorporation assay was then performed to evaluate their IC50 values.

BRAIN UPTAKE OF NEW ANTICANCER DRUGS

In situ brain perfusion in mice



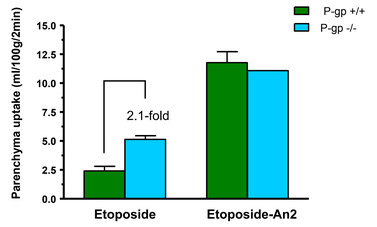
Brain uptake of the Etoposide-[125I]-An2 and Doxorubicin-[125I]-An2 was measured by *in situ* brain perfusion and compared to that of unmodified [3H]-Etoposide and [14C]-Doxorubicin

Transport Rate (Kin) of Anticancer Drugs in the Mice Brain

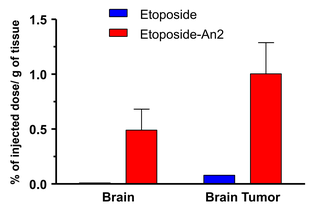
DRUG	BRAIN K_{in} (ml/s/g)
ANG1005	4.4×10^{-3}
Doxo-An2	3.7×10^{-3}
Etop-An2	1.4×10^{-3}
Paclitaxel	6.9×10^{-4}
Doxorubicin	2.8×10^{-4}
Etoposide	9.0×10^{-5}

Initial transport rate measured by in-situ brain perfusion in mice demonstrates that Angiopep-2 modified drugs are better transported than unconjugated drugs

Uptake of drugs in wild-type and P-gp deficient mice



Brain distribution 30 minutes after IV bolus



Brain uptake of Doxorubicin-[125I]An2 is identical in wild-type and P-gp knock-out mice indicated that it is not a P-gp substrate in contrast to [14C]etoposide

After i.v. bolus injection, Etoposide-[125I]An2 present a higher brain and brain tumors distribution than unmodified [14C]etoposide in mice intracranially implanted with glioblastoma U87 cells

CONCLUSIONS

- In addition to ANG1005 which shows promising results in Phase 1/2 studies (Abstracts:#3781 and #3782), two new anticancer etoposide and doxorubicin derivatives have been generated using the Angiopep- 2 peptide.
- Both new anticancer drugs :
  - Inhibit cancer cell proliferation *in vitro* with low IC_{50} values
  - Have a better brain penetration than unmodified drugs
  - Bypass the efflux pump (MDR1, P-gp)
  - Show a better brain and tumor distribution than unmodified drugs
- Work is on-going to evaluate their *in vivo* efficacy.

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Creating breakthrough drugs to treat brain diseases