Enhanced drug delivery to brain tumors with a new paclitaxel-peptide conjugate

 # 139

F. Bichat[1], M. Demeule[2], B. Lawrence[2], O. Raguin[1], B. Sourzat[1], R. Gabathuler[2], J-P. Castaigne[2], P. Genne[1]

[(1)] Oncodesign Biotechnology, Dijon, France ; [(2)] Angiochem Inc, Montreal - Quebec, Canada

ABSTRACT

Background: The main limiting factor in the treatment of brain tumors or metastasis is the low accessibility of the central nervous system (CNS) to drugs due to the blood-brain barrier. In the present study, the utilization of a new strategy based on a peptidic vector (Angiopep) capable of delivering drugs into the CNS in non-invasive manner was evaluated. Paclitaxel which accumulation into the CNS is hindered due to the P-glycoprotein efflux pump, was conjugated to the peptidic vector. The in vitro and in vivo properties of this conjugate (ANG1005) were characterized using different approaches.

Material and methods: The sensitivity of a panel of cancer cell lines to ANG1005 was evaluated in vitro. The pharmacokinetic behavior of ANG1005 in plasma after IP, IV injection or IV infusion and its toxicity were determined in vivo on healthy Nude rats. The antitumor activity of ANG1005 was evaluated by magnetic resonance imaging (MRI) in a model of Nude rats bearing NCI-H460 lung tumor implanted in the brain.

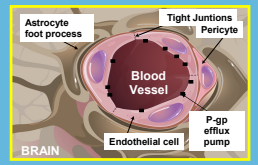
Results: Among all tumor cell lines tested in vitro, ANG1005 displayed an IC_{50} (concentration inducing a 50% cell death) in the nanomolar range for the NCI-H460 and U-87 MG cell lines. These IC_{50} were of the same order of magnitude than for paclitaxel. Toxicity experiments showed that the maximal total treatment dose (MTTD) using a Q3Dx5 schedule was 6 mg/kg/inj when ANG1005 was injected IV. With the same schedule, IV infusion enabled to increase treatment doses as the MTTD reached 15 mg/kg/inj. Pharmacokinetic studies indicated that maximal ANG1005 plasma concentrations were similar after a single IV injection at 11.25 mg/kg or an IV infusion at 15 mg/kg. However, the area under the time-concentration curve (AUC) was slightly higher for rats receiving ANG1005 via IV infusion as compared to rats dosed via IV injection. After a single IP injection at 75 mg/kg, ANG1005 plasma concentrations and AUC remained lower. Preliminary in vivo experiments were performed in models of Nude rats bearing NCI-H460 and U-87 MG tumors. MRI revealed a reduction of tumor growth early after the start of treatments for rats treated IP with ANG1005 at 75 mg/kg as compared to rats receiving the vehicle or paclitaxel.

Conclusions: These results demonstrate that ANG1005 delivers paclitaxel into the CNS and enhances its activity in an aggressive model. ANG1005 is currently under evaluation in phase I clinical trials for the treatment of glioma and brain metastases in human.

INTRODUCTION

The BBB is a unique, selective barrier formed by the endothelial cells that line the cerebral capillaries. These properties of the BBB are important as they provide an insulated environment for stable neuronal function.

Endothelial cells forming the BBB:
- Express tight junctions
- Lack fenestra
- Lack transendothelial channels
- Lack pinocytic vesicles
- High levels of the active efflux pump (P-gp)

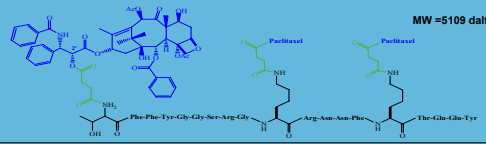


PEPTIDE VECTOR

Peptides	Amino acid sequence	Net charge
Angiopep-2	TFFYGGSRGKRNNFKTEEY	+2

ANG1005, an Angiopep-2 conjugated with 3 molecules of Paclitaxel

MW =5109 daltons



MATERIAL AND METHODS

In vitro experiments:
- Human tumor cell lines:
  - NCI-H460 (human lung carcinoma)
  - U-87 MG (human glioblastoma)
- Determination of the IC_{50} and GI_{50} of ANG1005 and paclitaxel using MTS and BrdU assays, respectively

In vivo experiments:
- Pharmacokinetic study of ANG1005 in healthy Nude rats:
  - Implantation of catheters in the femoral vein of rats
  - Single treatment with ANG1005: IP bolus injection at 75 mg/kg, single IV bolus injection at 11.25 mg/kg and single 4-hour IV infusion at 15, 20, 25 and 30 mg/kg
  - Collection of blood for plasma preparation on selected timepoints
  - Determination of ANG1005 levels in plasma using HPLC/MS
- Antitumor efficacy study in tumor bearing Nude rats:
  - Implantation of catheters in the femoral vein of rats
  - Intracranial injection of U-87 MG tumor cells on day 0 (D0)
  - Randomization on day 10 (D10) according to individual body weights
  - Treatments and monitoring (survival and body weights):
    - ➢ Experiment 1:
      - ✓ Vehicle (D10, D13, D17, D20) and ANG1005 (75 mg/kg/inj, D10, D13, D17) *via* IP route and Taxol® *via* IV *bolus* route (5 mg/kg/inj, D10, D13, D17, D20),
      - ✓ Evaluation of intracranial tumor volume by MRI for 3 out of 8 rats/group at D10 and D17
    - ➢ Experiment 2:
      - ✓ Vehicle (D10, D13) *via* 4-hour IV infusion, ANG1005 (6 mg/kg/inj, D10, D13) and Taxol® (5 mg/kg/inj, D10, D13) *via* IV *bolus* route
      - Evaluation of intracranial tumor volume by MRI for 4 out of 8 rats/group at D10 and D21

RESULTS

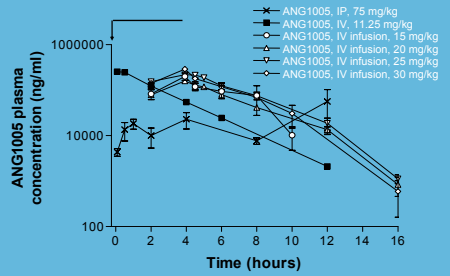
IC_{50} AND GI_{50} DETERMINATIONS

IC_{50} (nM)	NCI-H460	U-87 MG
ANG1005	13 ± 10	18 ± 11
paclitaxel	7 ± 3	13 ± 3

GI_{50} (nM)	NCI-H460	U-87 MG
ANG1005	6	25
paclitaxel	10	10

ANG1005 and paclitaxel exhibited an equivalent cytotoxic activity against the NCI-H460 human lung adenocarcinoma cell line and against the U-87MG human glioma cell line.

PHARMACOKINETIC STUDY



The arrow indicates IP or IV bolus injection. The bar indicates the IV infusion period

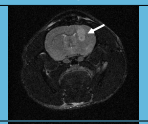
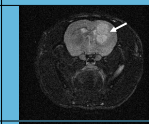
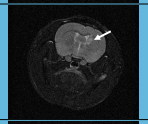
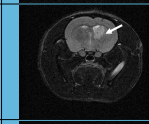
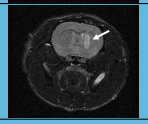
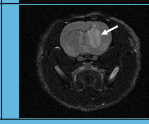
Treatment	AUC (µg/mL.h)	C_{max} (µM)
ANG1005, IV bolus, 11.25 mg/kg	731	50
ANG1005, IV infusion, 15 mg/kg	812	39
ANG1005, IV infusion, 20 mg/kg	736	31
ANG1005, IV infusion, 25 mg/kg	1,180	42
ANG1005, IV infusion, 30 mg/kg	1,160	57
ANG1005, IP bolus, 75 mg/kg	589	11

AUC: area under the curve
C_{max}: maximal ANG1005 plasma concentration

For IV bolus injection and IV infusion, the maximal plasma concentration was reached just after the injection and at the end of the infusion period, respectively.
For a single IV bolus injection at 11.25 mg/kg (dose corresponding to the MTD), C_{max} was higher than for a single IV infusion at 15 mg/kg (dose corresponding to the MTTD), but AUCs were of the same order of magnitude.
For IV infusion, no clear correlation was observed between treatment dose and C_{max} / AUC.
Although the treatment dose was increased, ANG1005 plasma concentration and AUC after a single IP injection remained lower than observed after a single IV bolus injection and IV infusion.

ANTITUMOR EFFICACY STUDY

Representative MRI Images of experiment 2

Treatment	D10	D21
Vehicle		
ANG1005		
Taxol®		

Tumor is indicated by a white arrow

Experiment 1	Mean tumor volume (mm^3)	
Treatment	D10	D17
Vehicle	8 ± 3 (n=3)	29 ± 16 (n=3)
ANG1005, IP, 75 mg/kg/inf	11 ± 2 (n=3)	7 ± 5 (n=3)
Taxol® IV bolus, 5 mg/kg/inj	9 ± 5 (n=3)	14 ± 9 (n=3)

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)

MR morphological images revealed that the mean brain tumor volumes were lower in groups treated with ANG1005 compared to free paclitaxel.

CONCLUSIONS

ANG1005 was as potent as paclitaxel to inhibit the in vitro growth of NCI-H460 and U-87 MG human tumor cell lines.
Biodistribution studies showed that the maximal plasma concentrations were about 1,000-fold higher than the in vitro concentration required to induce a 50% cell death.
Despite toxicity, preliminary antitumor efficacy studies revealed an early intracranial tumor growth inhibition after the treatments with ANG1005. These results need to be confirmed in further studies.
ANG1005 is currently under evaluation in two phase I studies in patients with brain cancer.