A new brain-penetrant Angiopep-2-morphine-6-glucuronic derivative (ANG2010) with angiogenic properties

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ABSTRACT

The blood-brain barrier (BBB) with tight junctions connecting brain capillary endothelial cells and high expression of active efflux transport proteins, plays a major role in central nervous system (CNS) homeostasis. The BBB serves as the natural gatekeeper of the brain, restricting entry of most pharmaceuticals while allowing essential molecules, such as glucose, insulin, and growth hormones to penetrate. Overcoming the obstacles posed by the BBB is a critical challenge for central nervous system (CNS) drug development. A new family of peptides derived from proteins that efficiently cross the BBB by low affinity receptor mediated transcytosis (LAMT) has been designed and is in early preclinical development for CNS delivery. This new engineered peptide compound platform promises higher levels of CNS exposure for therapeutic molecules. Here we report the development of a flexible platform creating new drugs which have access to the central nervous system using LRP for the treatment of CNS diseases.

In the present study, we explored the efficacy of ANG2010, a potential new of morphine, morphine-6-glucuronic acid (M6G). The results of this novel chemistry, ANG2010, was evaluated for brain uptake and efficacy in models of analgesia. Despite the fact that morphine and M6G, which are equi-effective after systemic administration, the analgesic potential of M6G has been shown to be 130-fold higher than morphine after intracranial injection. However, the brain penetration of M6G is significantly lower than morphine, thus limiting its utility in pain management. Using an in vivo mouse paradigm, we obtained a higher rate of brain penetration for the new chemical entity, ANG2010 compared to that of unencapsulated M6G and morphine. This increase in brain uptake results in a significant improvement in the pharmacological efficacy of M6G in the mouse acute pain and rat tail flick assays. ANG2010 administration in a pain model significantly increased paw flick latency for at least 3hrs.

INTRODUCTION

Angiogenics is a clinical-stage biotechnology company discovering and developing new breakthrough drugs that are uniquely capable of crossing the blood-brain barrier (BBB). The BBB is, in essence, a unique and specialized barrier. These molecules cannot be effectively addressed 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 insulating 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 transepithelial channels.
- Lack pericyte mediated activity.
- Express high levels of the active efflux pump (P-gp).

Angiogenics proprietary DPC technology targets the low-density lipoprotein receptor-related protein (LRP) receptor family. This endogenous transmembrane protein has a number of inherent biochemical advantages for drug transport across the BBB, including high affinity binding, low toxicity, and a broad range of binding specificity. ANG2010, a new regulatory molecule containing the morphine-6-glucuronide (M6G) coupled to an Angiopep-2 (Angiopep-2-M6G) was shown in the preclinical setting to be an effective pain treatment agent. In the present study, we investigated the brain uptake and analgesic effects of a new chemical entity formed by conjugation of M6G to Angiopep-2 in mice and rats that crosses the BBB. Results of in vivo brain penetration in mice demonstrated that the Angiogenics ANG2010 compound was able to penetrate the BBB of mice and rats, with significant pain reduction.

METHODS

1. Evaluation of in vivo brain uptake:

- **Animals:** Male Wistar rats (250-300 g) were housed individually in a temperature- and humidity-controlled environment. Rats were fasted for 12 hours prior to sacrifice and then anesthetized with sodium pentobarbital (30 mg/kg, IP) and allowed to recover for at least 30 minutes before use.

- **Peptide administration:** ANG2010 (200 μg, IP) was administered to rats after anesthesia. At selected time points (5 min, 30 min, 60 min, 120 min), the rats were sacrificed and the brain samples were collected for in vivo brain uptake studies.

- **In vitro brain uptake:** Brain tissues were homogenized and assayed for peptide levels using a specific ELISA kit.

2. Evaluation of analgesic effect in pain models:

- **Hot plate mouse model:** Male rats were placed on a hot metal plate maintained at 48°C and pinna reflex response was measured after a period of 30 minutes. Morphine was given subcutaneously. The time to remove the tail was measured, with a maximum time of 60 seconds.

- **Tail flick reflex model:** Pain threshold was measured before baseline and after drug administration, using a standard hot water tail-flick assay. The dependent variable was the latency (in seconds) for the rat to flick its tail from the hot water bath. The water temperature was maintained at 50°C for the temperature pain test. Each tail was immersed in the bath to reach the baseline latency, and the test time required for the rat to remove its tail was measured. A statistically significant increase from baseline pain threshold measurement was interpreted as induction of analgesia.

CONCLUSIONS

- In addition to the anticancer agent, GRN1005, which shows promising results in Phase 1/2 studies for brain tumors, new Angiopep-2-M6G (ANG2010) has been generated for pain.

- In the present study, the main objective was to broaden the EPC platform for morphine derivatives by generating a new Angiopep-2-M6G derivative (ANG2010) for pain.

- ANG2010 crosses the BBB more efficiently than native morphine and M6G binds to opioid receptors with affinity similar to that of native morphine. Demonstrates in vivo efficacy (4% or 6% in two rodent pain models: Hot plate mouse model and rat tail flick model.

- Strong validation of the EPC platform for small molecules opens new avenues for other potential pain compounds that do not cross the BBB.