

Short communication

PACAP glycosides promote cell outgrowth *in vitro* and reduce infarct size after stroke in a preclinical model

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Abstract

Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) is a pleiotropic peptide known to promote many beneficial processes following neural damage and cell death after stroke. Despite PACAP's known neurotrophic and anti-inflammatory properties, it has not realized its translational potential due to a poor pharmacokinetic profile (non-linear PK/PD), and limited Blood-Brain Barrier Penetration (BBB) permeability. We have previously shown that glycosylation of PACAP increases stability and enhances BBB penetration. In addition, our prior studies showed reduced neuronal cell death and neuroinflammation in models of Parkinson's disease and Traumatic Brain Injury (TBI). In this study we show that a PACAP₍₁₋₂₇₎ glucoside retains the known neurotrophic activity of native PACAP₍₁₋₂₇₎ *in vitro* and a 5-day daily treatment regimen (100nM) leads to neurite-like extensions in PC12 cells. In addition, we show that intraperitoneal injection of a PACAP₍₁₋₂₇₎ lactoside (10mg/kg) with improved BBB-penetration, given 1-hour after reperfusion in a Transient Middle Cerebral Artery Occlusion (tMCAO) mouse model, reduces the infarct size after the ischemic injury in males significantly by ~36%, and the data suggest a dose-dependency. In conclusion, our data support further development of PACAP glycopeptides as promising novel drug candidates for the treatment of stroke, an area with an urgent clinical need.

Section snippets

Significance Statement

The Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) glycoside analogs, with improved stability and ability to cross the blood-brain barrier, described herein retain the native peptides known neurotrophic activity *in vitro* and we have shown that systemic administration of a PACAP lactoside reduces infarct size in a standard mouse stroke model by ~36%, indicating a neuroprotective effect, highlighting the potential of PACAP glycosides as novel drug therapy candidates for stroke. ...

Material

The glycopeptide synthesis (2LS80Gluc, 2LS80Lact) was performed by the Polt Laboratory using solid phase synthesis according to [8]. ...

PC12 cell culture

Undifferentiated PC12 cells were cultured as detailed prior [14], in RPMI media (cat# 11875135, Thermo Scientific, Waltham, MA) with 5% heat inactivated fetal bovine serum, 10% horse serum, 10µg/mL penicillin and streptomycin, on Poly-D-Lysine coated plates (density: 1.5×10^5 cells/well). 48-hours post-plating cells were treated with either vehicle (water), ...

Glycosylated PACAP₍₁₋₂₇₎ stimulates neurite outgrowth in PC12 cells

PC12 cells were treated and allowed to grow in culture for 5 days (Fig. 1A). At this time point untreated cells begin to form clusters as they divide and replicate, but do not typically have neurite projections (Fig. 1B). After 5 days of treatment the number of cells per frame was decreased relative to the group (108 ± 32.62 , PACAP₍₁₋₂₇₎ = 5.5 ± 2.62 , $p = 0.0002$) and PACAP₍₁₋₂₇₎ glucoside (7.11 ± 3.37 , $p < 0.0001$, vs vehicle) (Fig. 1C). The average cell diameter was increased after ...

Discussion

We have previously shown that glycosylated PACAP has improved PK/PD properties, compared to its native counterpart, thereby effectively turning PACAP into a “druggable” peptide [8]. The results of the present study indicate that the glycosylated PACAP analogs retain the well-described neurotrophic and neuroprotective properties of the native peptide and functional activity is retained *in vitro*, and are contributing to a protective effect in the context of ischemic stroke. ...

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CRedit authorship contribution statement

Kelsey Bernard: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Denali Dickson:** Methodology, Investigation. **Bobbi L. Anglin:** Resources, Methodology, Investigation. **M. Leandro Heien:** Supervision, Resources. **Robin Polt:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Helena W. Morrison:** Writing – review & editing, Supervision, Resources, Methodology, Investigation. **Torsten Falk:** Writing – review & editing, Writing – ...

Declaration of Competing Interest

MLH, TF, and RP hold patents related to the content. MLH, TF, and RP have equity in Teleport Pharmaceuticals, LLC, a UArizona biotech startup. This interest played no role in the design of the studies; in the collection, analyses, or interpretation of data; in the writing of the Paper, or in the decision to present the results. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict ...

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