PEPTIDE DELIVERY

YI-P 080 Delivery of Tailored Analgesic Peptide-drug Nanocontainers Across the Blood Brain Barrier

P. Anand[a], A. O'Neil, E. Lin[a], T. Douglas[b], M. Holforda
a Hunter College CUNY and The American Museum of Natural History, 695 Park Ave, NY, NY 10065, USA; b Montana State University, Bozeman, Montana, 59717, USA

Due to side effects associated with opioid drugs, nonopioids have been pursued to discover novel analgesic therapies. Ziconotide (Prialt®), discovered from Conus magus, is the first non-opioid marine snail drug approved for treatment of chronic pain in cancer and HIV patients. Despite being a breakthrough drug, widespread application of ziconotide is limited due to its size (25 amino acids), and lack of efficacy when delivered via common routes, an intrathecal delivery is required as ziconotide does not cross the blood brain barrier (BBB). Presented here is a strategy for alternative to intrathecal application of ziconotide by encapsulating it in a viral capsid nanocontainer for delivery across the BBB. Viral capsids, such as the *Salmonella typhimuriam* bacteriophage P22 capsid, are ideal encapsulation vehicles as they can be made in large quantities, have constrained interior spaces amendable for assembly and packaging bioactive cargo, and have large surface areas that are conducive to multivalent genetic and chemical modifications. Specifically, the interior of P22 capsid was modified with the sequence of ziconotide (MVIIA) at the Cterminus of the P22 scaffold protein. The exterior of the P22-MVIIA construct was chemically modified via bioconjugation of fluorescein labeled HIV-TAT peptide using free thiol groups on the P22 surface. HIV-TAT peptide has been shown previously to translocate several cell types and successfully enabled the engineered P22-MVIIA capsid to cross a BBB mimic of rat brain microvascular endothelial cell line. These results highlight a plausible strategy for delivering peptide drugs to their site of action using viral nanocontainers.

YI-P 081 Cleaved Intracellular SNARE Peptides are Implicated in a Novel Cytotoxicity Mechanism of Botulinum Serotype C

Jason Arsenault[a], Sabine AG Cuijpers[a], Enrico Ferrari[a,b], Dhevahi Niranjan[a], John O'Brien[a], Bazbek Davletov[a,c]
a MRC Laboratory of Molecular Biology, Neurobiology, Cambridge CB2 0QH, UK; b University of Lincoln, School of Life Sciences, Lincoln LN6 7TS, UK; c Department of Biomedical Sciences, University of Sheffield, Sheffield S10 2TN, UK

Recent advances in intracellular protein delivery have enabled more in-debt analyses of cellular functions. A specialised family of SNARE protease, known as Botulinum Neurotoxins, block neurotransmitter exocytosis, which lead to systemic toxicity caused by flaccid paralysis. These pharmaceutically valuable enzymes have also been helpful in the study of SNARE functions. Nevertheless, there have been recent reports of an ensuing cytotoxic effect by Botulinum serotype C. This enzyme cleaves intracellular SNAP25, as does serotype A and E, but also, exceptionally, cleaves Syntaxin1. Using an array of lipid and polymer transfection reagent we were able to deliver different combinations Botulinum holoenzymes into the normally unaffected, Neuro2A, SH-SY5Y, Pc-12, and Min6 cells to analyse the individual contribution of each SNARE protein and their cleaved peptide products. Our results show that the freely release Syntaxin and Syntaxin plus Synaptorbrevin peptides, which can still form SNARE complexes, wreck havoc on the intracellular trafficking machinery. These results were further confirmed by the direct cellular penetration of these SNARE peptide fragments potentiated by the very same transfection reagents. The freely diffusible Syntaxin fragments were shown to cause cytotoxic effects in most cell types and inhibited neurite outgrowth in differentiated Pc-12 cells. The treatment of ex-vivo cortical cells with the holoenzymes and SNARE peptides, showed a parallel cytotoxic effect and an observable Wallerian degeneration. These results open the door for a dual-acting pharmacological compound that would be used simultaneously as a secretion inhibitor and a cytotoxic moiety for the localised treatment of neuroendocrine tumors.

YI-P 082 Use of Peptide Technology for Analgesic Brain Delivery

É. Besserer-Offroy[1], M. Demeule[2], N. Beaudet[1], A. Régina[2], P. Tétreault[1], K. Belleville[1], C. Ché[2], A. Larocque[2], J.E. Lachowicz[2], J.P. Castaigne[2], and P. Sarret[1]
1 IPS, Université de Sherbrooke, Sherbrooke; 2 Angiochem Inc., Montréal, QC, Canada

The blood-brain barrier (BBB) is the most significant obstacle to effective CNS drug delivery. To overcome this challenge, we created new chemical entities that are transported across the BBB via the low-density lipoprotein related receptor (LRP1). Based on sequences from LRP1 ligands, a family of peptides (Angiopeps) has been designed to facilitate brain uptake. Here, we synthetized a new chemical entity by conjugating the Angiopep-2 (ANG) peptide with the 13aa neuropeptide, neurotensin (NT). We demonstrated by mouse in-situ brain perfusion that the ANG-NT (ANG2002) derivative is transported at least 10-fold more efficiently across the BBB than is native NT. *In vitro*, ANG2002 binds to both NT receptors (NTS1 and NTS2) and conserves the biological activity of native NT. *In vivo* studies demonstrate that in rats, ANG2002 administered intravenously induces a dose-dependent analgesia in both phases of the formalin model of persistent pain. At a dose of 0.05 mg/kg, ANG2002 was also effective in reversing pain behaviors induced by chronic constriction injury of the sciatic nerve (neuropathic pain) or femoral inoculation of MRMT-1 rat breast cancer cells (bone cancer pain). Overall, these data extend the validation of Angiopep-2 conjugation to include neuropeptides such as NT, and establish the benefits of Angiochem's technology for the development of new CNS therapeutics.

YI-P 083 A Model System for Controlled Drug Release in Breast Cancer Using Cleavable Fluorophore-Neuropeptide Y Conjugates

D. Böhme and A. G. Beck-Sickinger
Universität Leipzig, Institute of Biochemistry, Brüderstraße 34, 04103 Leipzig, Germany

The human Y1-receptor subtype was found to be overexpressed in more than 90% of breast cancer patients and in 100% of breast cancer derived metastases.[1] Binding of the natural ligand neuropeptide Y (NPY) to human Y-receptors leads to receptor-mediated endocytosis. Using Y1-receptor subtype selective NPY analogs permit specific delivery of attached cargoes to breast cancer cells.[2] In order to release these compounds in a selective and controlled manner after internalization various linker structures can be used, which enable cleavage by enzyme, pH or reduction induced mechanisms. This approach allows an efficient intracellular delivery of drugs or imaging agents.

This work combines the breast cancer cell selective delivery system using the Y1-receptor subtype selective [F7, P34]-NPY with the controlled intracellular release of fluorescent dyes attached to the peptide by different cleavable linkers. Single and double fluorescently labeled NPY analogs were synthesized by Fmoc/t-Butyl solid phase peptide synthesis. Fluorescence microscopy internalization studies using live cell imaging revealed the intracellular cleavage of linker structures and release of fluorophores. The model system shows the potential of this smart delivery approach to selectively target breast cancer cells with cytostatic agents.