Novel long-acting analgesic for chronic pain.

Prof Bazbek Davletov, The University of Sheffield, UK

Introduction

Botulinum neurotoxin type A (BoNT/A) can induce analgesia in a number of chronic pain conditions (1,2) in addition to its more well-known paralytic action at the neuromuscular junction (3). The long-lasting nerve blocking effect can last up to 6 months in humans (4) and therefore BoNT/A represents an attractive candidate to develop a new approach to treatment of chronic pain. Our lab is currently investigating new technologies in order to re-engineer BoNT so that the long-lasting nerve-blocking actions remain but the molecule is re-targeted away from the neuromuscular junction toward sensory and nociceptive neuronal populations. In order to develop new chronic pain therapeutics, in collaboration with colleagues at Leeds University, we have engineered a novel molecule combining the enzymatic part of BoNT/A with a neuron-targeting cholera toxin subunit B (CTB) termed **ChoBot** (Fig 1). The cholera toxin B subunit (CTB) binds to cellular surfaces via its receptor monosialoanglioside (GM1), where it is internalised and transported into neurons. In normal conditions, the GM1 ganglioside is present in a subpopulation of myelinated sensory neurones. However, following nerve injury it has been reported that a phenotypic switch can result in more nociceptive afferents taking up the cholera toxin (5,6). Therefore, by utilising our unique technologies and targeting nerve blocking botulinum molecules towards more specifically pain-sensing neurones rather than motor neurones, we hope to retain the long-lasting effects of native BoNT catalytic action but reduce their associated highly paralytic effects and therefore produce a more desirable and safer novel analgesic.

Specifically, the aim of this project were to:

1. Investigate the efficacy of a novel bioengineered botulinum molecule and ascertain its long lasting effects free from paralysis in animal models of chronic neuropathic pain.
2. Analyse biodistribution of ChoBot-cleaved SNAP25 expression in peripheral and central tissues in animal models of pain using immunohistochemistry
3. Establish in vitro characterisation of ChoBot targeted cells

Results to date

To detect functional ChoBot activity both in vitro and in tissue sections, we used a cleaved SNAP25 polyclonal antibody (developed in house) which recognises the botulinum-cleaved end of the SNAP25 protein. We found that the novel ChoBot molecule is able to efficiently cleave SNAP25 in some dorsal root ganglion neurones with the uptake and cleavage of SNAP25 being predominantly targeted toward myelinated neurones. We also show similar results within neuronal populations in the epidermis following injection of ChoBot into rodent glabrous skin.

To determine the safety of the ChoBot molecule, we also examined its effect on motor function using electromyography and measured compound muscle action potentials in the gastrocnemius muscle following subcutaneous injection of ChoBot at varying doses. Compared with the native BoNT/A, ChoBot is significantly less paralytic at 100x higher doses indicating that it is not able to enter the neuromuscular junction. Further immunohistochemistry experiments are underway to investigate the presence of cSNAP25 and co-localisation with αbungarotoxin, a neuromuscular junction marker.

We have also set up a number of animals for the chronic pain models we are investigating, the spared nerve injury model (SNI) and chemotherapy-induced polyneuropathy model (CIPN). To date we have completed behavioural results from control animals and animals injected with vehicle for both SNI and CIPN pain models. We show here (Fig 1) that we can elicit a robust pain phenotype in animals undergoing these procedures and behavioural analysis using the von Frey monofilament system and Up-down method is producing consistent mechanical hypersensitivity responses.

1. Safarpour & Jabbari. Toxicon 2018; 147:120-8
2. Attal et al. Lancel Neurol 2016; 15:555-65
3. Simpson. Pharmacol Rev 1981; 33:155-88
4. Eleopra et al. Mov Disord 2004;19:S53-9
5. Shehab etal. Brain Res. 2003, 964, 218–227.
6. Tong et al. J. Comp. Neurol. 1999, 404, 143–158.

Fig1. Baseline data indicating effectiveness of both the spared nerve injury model and chemotherapy-induced neuropathy model to induce mechanical sensitivity