Novel long-acting analgesic for chronic pain

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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 collaboration with colleagues at Leeds University, we engineered a novel molecule combining the enzymatic part of the native BoNT/A with a neurone-targeting Cholera toxin subunit B termed **ChoBot**. The cholera toxin B subunit 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.

We use a combination of biochemical techniques, in vitro cell biology, in vivo immunohistochemistry and analgesic studies in rat pain models to reveal analgesic potentials of novel, engineered botulinum molecules. We have shown that the ChoBot molecule is able to efficiently cleave SNAP25 protein in a subset of dorsal root ganglion neurones in cultures and within neuronal populations in the epidermis following injection of ChoBot into the glabrous skin of the rat hind paw.

Our recent results, in which we used a cleaved SNAP25 polyclonal antibody (developed in house) which recognises the botulinum cleaved end of the SNAP25 protein have enabled us to establish further the specific populations of peripheral neurones targeted by the molecule. Injection of ChoBot into the subdermal skin results in the observation of cSNAP25 in a large population of fibres within the dermis projecting toward the epidermis. Quantitative analysis showed these fibres were predominantly NF200 myelinated fibres. A smaller population of Calcitonin gene-related peptide (CGRP) primary afferents also showed co-localisation with the cSNAP25 signal (Fig 1a). These results indicate that it is possible to re-target botulinum molecules away from their natural target at the neuromuscular junction by combining with the Cholera molecule. Additionally, we show that, in the same animals, cleaved SNAP25 is observed in the cell bodies of smaller myelinated peripheral neurones to potentially indicate that the ChoBot molecule undergoes uptake at the peripheral terminal and is transported centrally (Fig 1b).

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 targeted to the neuromuscular junction. Further immunohistochemistry experiments are underway to investigate the presence of cSNAP25 and co-localisation with αbungarotoxin, a neuromuscular junction marker.

Our current efforts are now mainly centred on demonstration of analgesic action following local injections in rats with neuropathic pain conditions.

Figure 1

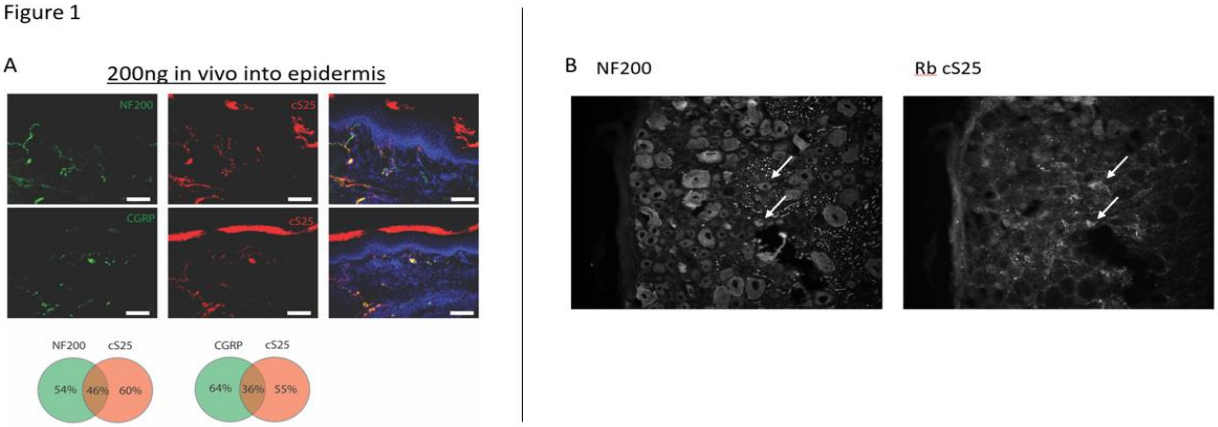

Figure legend. (A) Partial co-localisation of botulinum-cleaved SNAP25 with neuronal markers Neurofilament 200 and Calcitonin gene-related peptide (CGRP) was revealed by immunohistochemistry of ChoBot-injected glabrous skin of the rat hind paw. (B) Immunohistochemistry of the ipsilateral dorsal root ganglion reveals a small subpopulation of sensory neurons carrying the botulinum-cleaved SNAP25 which suggest a central action of the ChoBot molecule.

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