SOCIETÀ ITALIANA DI FARMACOLOGIA

RELAZIONE DI METÀ PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: SIF/MSD-ITALIA

TIPOLOGIA DI RELAZIONE: METÀ PERIODO

TITOLO DELLA RELAZIONE: NEW NON-PARALYTIC BOTULINUM MOLECULES FOR THE CONTROL OF PAIN

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RELAZIONE:

In both animals and humans life events such as accidents or surgical interventions can lead to uncontrollable pain that can persist long after the original injury. This is chronic pain, the treatment of which is yet to be resolved – 19% of adult Europeans suffer from chronic pain of moderate to severe intensity; less than 60% receive adequate pain relief [1]. New treatments are required but to develop these requires new therapeutic approaches and a better understanding of what goes wrong in the pain pathways resulting in uncontrollable pain.

Tetanus and botulinum toxins (TeNT and BoNT), produced by anaerobic bacteria of the genus Clostridia are a dangerous threat to human health; causing pathologies ranging from food poisoning to gangrene [2]. They are two of the most dangerous toxins known to man, just 0.09-0.15 µg of intravenous or intramuscular Botulinum neurotoxin type A (BoNT/A) is lethal to a 70 kg human [3].
The toxins have three domains with specific functions. Two heavy chains, HN¬ and HC, are responsible for membrane translocation and neurospecific binding respectively. A disulphide bridge connects the HN chain to the light chain (Lc), or silencing domain, a zinc endopeptidase that cleaves members of the SNARE family responsible for neuroexocytosis; synaptobrevin, SNAP-25 and syntaxin [4]. Cleavage of these proteins prevents neurotransmitter release and causes paralysis.
In vivo, tetanus and botulinum toxins cause differing paralysis due to the neurospecificity of their HC binding domain, and thus their site of action [5]. When injected peripherally, BoNT heavy chain binds to its specific receptor on the neuronal membrane, is endocytosed, translocates to the cytoplasm, and blocks release of acetylcholine from the neuromuscular junction (NMJ), causing flaccid paralysis at its site of entry [4]. Tetanus, on the other hand, has a HC that enters non-acidified carriers, thus preventing peripheral light chain translocation, and is sorted it into the motor neuron retrograde transport system and take it to the central nervous system. The TeNT transport rate to the motorneurone is approximately 7.5 mm per hour [6]. Once transported through the retrograde microtubule pathway, the heavy chain of tetanus allows it to be transcytosed to inhibitory interneurons, silence these inhibitory interneurons, and result in spastic paralysis [4].

However the group at UCL and Sheffield has found a way to separate the paralysing effects of the toxin from its silencing effects on other neurons. By using this new synthetic chemistry in this project I am trying to target and silence specific populations of neurons reversibly and alleviate chronic pain states.

The experiments outlines in this application will allow us to move rapidly towards the clinic and to apply these new botulinum derivatives to different types of chronic pain. I'm now evaluating these new molecules in rodent pain models and adjusting their potential for targeting specific pain mechanisms. I have two major research questions. First, do non-paralytic botulinum molecules (BiTox) alleviate hypersensitivity in common models of pain: neuropathic, inflammatory and surgical? Second, is it possible to retarget botulinum molecules (conjugated to tetanus binding domain, SP or other neuropeptides) to other neurons involved in pain circuitry? It was recently demonstrated that botulinum neurotoxin could be split into two fragments (SNAP25 protease and the targeting domain) that can then be either rejoined or redirected to specific cells using new technology [7; 8]. Importantly, the re-assembled binary neurotoxin exhibits no systemic toxicity and is one thousand times less potent at the neuromuscular junction than the native toxin. The lab in UCL now has non-paralytic botulinum molecules that I'm testing in well-established animal pain models to ascertain whether silencing of sensory neuronal pathways can be achieved without muscle-related effects and that direct application of the conjugates to the spinal cord will target specific pain sensing neurons.

As part of my research activity in London I'm using three pain models, previously described [9-12], and used in the screening of antinociceptive drugs: i) Inflammatory pain models. Inflammation is induced by injection of Complete Freund's Adjuvant (CFA, 10 µl) or carageenan into the plantar surface of the hindpaw or in the left ankle joint, under isoflurane anaesthesia. I measure both mechanical and thermal hypersensitivity. Weight bearing and von Frey hairs are used to monitor mechanical sensitivity. ii) Incisional model of surgical pain. Mechanical threshold at the central plantar surface of the left hind paw is assessed before surgery (as basal threshold) and then testing from 4 h after incision. Botulinum constructs are injected locally before or after incision and mechanical pain thresholds measured with von Frey hairs. iii) Neuropathic pain models: Spared nerve injury (SNI) is used. Behavioural testing begins 48 h after surgery and continued for up to 21 d postsurgery. In some cases and to mirror more closely the clinical situation, botulinum constructs will be given intrathecally after SNI neuropathy has developed and changes in thresholds monitored.
Following completion of the experiments tissue from spinal cord DRG and brain will be taken fresh for Western Blot analysis to measure levels of cleaved SNAP25 (a marker of BiTox activity) or perfused for immunohistochemical analysis again using botulinum-cleaved SNAP25. Crucially I need to know if BiTox is effective in reducing pain behaviour in the pain models and also if we can introduce the silencing domain of the botulinum molecule into neurons by virtue of a surface receptor such as NK1 or the opiate receptor.
Ultimately, I can envisage pilot trials of these constructs in patients receiving palliation for severe pain.

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