Targeted Glucocorticoids for Localized, Chronic Pain: Preclinical Feasibility for Non-Opioid Pain Medication

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Can we treat localized, chronic pain with a self-injectable pen that delivers & "targets" a potent glucocorticoid to the Dorsal Root Ganglia?

As of August 2018, of the estimated 51,000 troops wounded in OIF/OEF/OND almost half continue to experience chronic pain ("55.6% reported nearly daily or constant frequency").[1,2] Of the 2.8 million who have deployed since 9/11, up to 500,000 Wounded Warriors and Veterans may be at risk of substance abuse because they have been, or will be prescribed opioids to treat their pain.[3]

With support from DOD Log 1327104, we have shown Proof-of-Concept (POC) *in vivo* of two intraneuronally-targeted test articles: fluocinolone acetonide (FA) bioconjugated to recombinant human Nerve Growth Factor, where rhNGF is known to localize at the site of peripheral injection for tropomyosin kinase receptor A (TrkA) mediated retrograde axonal transport to the Dorsal Root Ganglia (DRG); and to brain-derived neurotrophic factor (rhBDNF), for TrkB-medicated absorption by, and intraneuronal delivery to central neuronal cell bodies. This well-established principle of "differential distribution"[6] is illustrated in Figure 1:

Sub-c FA-NGF binds to TrkA receptors on sensory neurons at periphery. FA-NGF is delivered intraneuronally via retrograde axonal transport to DRG.

Sub-c FA-BDNF binds to TrkB receptors on alpha motor neurons at periphery. FA-BDNF is delivered intraneuronally via retrograde axonal transport to CNS.

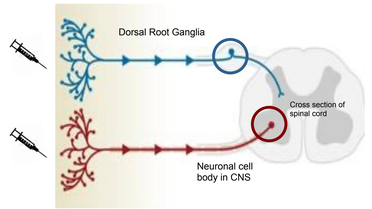


Figure 1. Differential Distribution

NGF (BDNF) is selective for TrkA (TrkB) receptors expressed on distal ends of sensory (alpha motor) neurons. When injected into the periphery, neurotrophins are absorbed selectively and delivered to the neuronal cell body.

1. When NGF is injected at the periphery, it is absorbed by and internalized into sensory neurons due to NGF's high affinity for TrkA receptors expressed on the distal ends of sensory neurons. The NGF-TrkA complex is moved via retrograde axonal transport to the neuronal cell body, to dorsal root ganglia (DRG), where it degrades.

2. When BDNF is injected at the periphery, it is absorbed by alpha motor neurons due to BDNF's high affinity for TrkB receptors expressed in the periphery at the distal ends. The BNDF-TrkB complex is moved via retrograde axonal transport to the neuronal cell body in the spinal cord, where it degrades.

Case for intraneuronal delivery of fluocinolone acetonide:

- Increased potency reduces dose of rhNGF (rhBDNF)
- "Fire and forget" - low to no risk of substance abuse or diversion
- Localized "targeted" delivery may improve dose-limiting toxicities

First discovered in 1925 to treat sciatica,[7] glucocorticoids (GCs) are now understood to act intracellularly by binding to Glucose Response Elements. When injected into the dorsal horn for treatment of sciatica or postherpetic neuralgia, GCs can be 60% effective.[8,9] Glucocorticoids are potent inhibitors of pain because they act on multiple molecular targets including the key pro-inflammatory cytokines IL-1β, IL-6, and calcitonin-related peptide; and the anti-inflammatory IL-10; prostaglandins; and cyclo-oxgenases (COX-1, COX-2).[10]

We selected FA, which is FDA approved for intravitreal inserts, as drug payload because it is ~20X more potent than methylprednisolone. By increasing potency of payload, preliminary results suggest that we can reduce the absolute amount of neurotrophin to levels known to be well tolerated in the clinic (for NGF, Genentech's dosing in Phase 2 clinical trials[11]; for BDNF, Amgen's dosing in Phase 2 clinical trials[12]).

Fig 2. Relative potency (Structure Activity Relationship) Among Glucocorticoids

Preclinical Data: FA-rhNGF exerts strong effect in electrophysiology studies of chronic SCI pain (Fig. 3); FA-rhNGF and FA-rhBDNF show rapid onset in acute pain, but limited durable analgesic effect (Figs. 4, 5); these results are consistent with previous data in a model of chronic pain (Varicella zoster virus, "shingles") (Fig. 6)

Approach. All studies done under DOD Log 13273014 were in rat, in which neurotrophin/Trk relationship per dermatome recapitulates the human neuroanatomy. We tested FA-rhNGF and FA-rhBDNF in two established models of pain, in the Spinal Nerve Axotomy (SNA) model of peripheral pain, and in the T10 model of spinal cord injury (SCI) pain. Study articles (FA-rhNGF, FA-rhBDNF, 800-rhNGF, 800-rhBDNF) were well characterized using standard analytics (RP-HPLC) before being plated in primary neuronal cultures to determine survival (bioassay for TrkA/NGF in the Lein lab, in superior cervical ganglia; bioassay for TrkB/BDNF in the former Barres lab, Stanford, in Retinal Ganglion Cells) (data not given). We measured pain with an established behavioral metric, von Frey hairs (VFH) to measure mechanical allodynia, and with Mechanical Conflict, to measure aversion to walking on sharp probes.

Figure 3. In electrophysiology studies, Fig. 3 shows a profound effect of FA-rhNGF in neurons from the SCI pain model.
Figure 4. Intrathecal (L5) FA-rhNGF (**blue line**) shows rapid onset, but a modest, non-durable effect (Days 0, 1, 2 and 3 QD, 6 µg FA-rhNGF).
Figure 5. In the same study as in Fig. 2, we assessed pain sensitivity using the Mechanical Conflict ("Coy box") behavioral metric (**fewer crosses = more pain**). Analgesic effect for FA-rhNGF and FA-rhBDNF is more pronounced at the higher (3 mm) probe.
Figure 6. Results from Figs. 2, 3 and 4 confirm earlier data shown in Fig. 5. In a model of chronic pain (Varicella zoster virus model, in rat), FA-murineNGF given intramuscularly shows rapid onset and durable analgesic effect after 4 IM injections 1X-daily (QD) of 0.34µg FA per dose (5µg NGF per dose).
Additional data not shown: Both probes 800-rhNGF, 800-rhBDNF showed that NGF (BDNF) localized 800 dye when injected at the periphery.

Fig. 3. Electrophysiology studies: FA-rhNGF

FA-rhNGF significantly reduces ongoing activity and hyperexcitability after spinal cord injury in a model of Chronic, Spinal Cord Injury Pain. One month or longer after contusive spinal cord injury at T11, we excised dorsal root ganglia below the injury level, dissociated the neurons, treated the cultures with 220nM nM FA-rhNGF overnight, and tested the isolated sensory neurons on measures of hyperexcitability we know to be important for driving ongoing activity (OA) and ongoing pain after SCI. The isolated sensory neurons are probably nociceptors because the same type of sensory neurons respond to capsaicin and bind IB4.

FA-rhNGF had a profound suppressive effect on all measures related to ongoing activity and excitability in dissociated nociceptors. Significant reduction was found in SA at rest, ongoing activity (OA) at -45 mV, Action potential (AP) voltage threshold, and AP current threshold (rheobase). Such effects have never been described for glucocorticoids or for NGF, which should have the opposite effect.

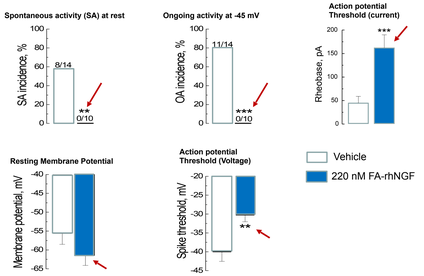


Fig. 4. (von Frey Hairs) Intrathecally administered (L5) FA-rhNGF may reduce mechanical allodynia in SNA rats

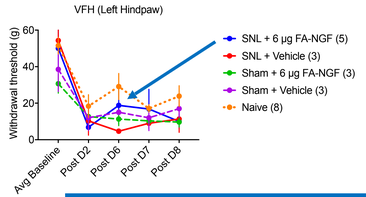


Fig. 5. (Mechanical Conflict) Intrathecally administered (L5) FA-rhBDNF and FA-rhNGF may reduce mechanical allodynia and hyperalgesia in SNA rats

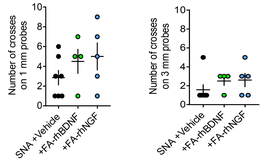


Fig. 6. (von Frey Hairs) FA-murineNGF is analgesic in a model of chronic pain (post-herpetic neuralgia)[13]

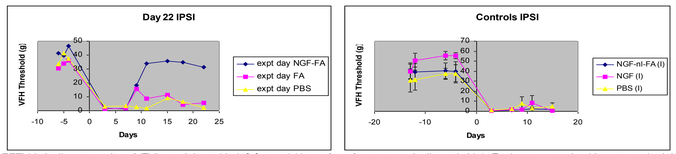


Fig 6. (6-LEFT) Varicella zoster virus (VZV) was injected in left footpad (day = 0; n=6 per group, ipsilateral side). Each group received intramuscular injections days 7-10 of (1) NGF-drug conjugate, (2) equivalent unmodified drug, or (3) PBS. VZV Infection produced a severe allodynia. VFH threshold dropped from ~ 35 g to 1-3 g (VFH). FA-murineNGF alleviated allodynia within 3 days; effect was sustained (Significance: P<0.0079 for NGF-FA vs FA; P<0.0079 for NGF-FA vs PBS; P<0.15 for FA vs PBS.) To confirm the role of linker chemistry, *ie* rule out non-specific binding of FA to NGF, we admixed these two components and administered them. **(6-RIGHT)** Unconjugated FA showed almost no activity compared to the conjugated GC variant.

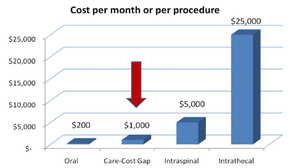
Conclusions: Develop for Chronic Pain

Conclusions. From the preliminary data, we conclude that our multi-part hypothesis has been confirmed, that (1) neurotrophins can deliver GCs intraneuronally; (2) localized dosing may fall within established clinical safety parameters for FA, rhNGF, and rhBDNF; and (3) the analgesic effect is more pronounced and more durable in chronic, rather than acute or subacute pain.

Unmet need. There is an urgent unmet clinical need to treat chronic pain effectively, but with an improved safety profile, with a lower risk of: (i) diversion and substance abuse; (ii) the use of opioids to attempt suicide[4]; (iii) unintended death due to respiratory suppression; and (iv) cognitive impairment, particularly when pain is co-morbid with Traumatic Brain Injury (TBI) and/or post-traumatic stress.[5]

Impact. We address a gap in Stepped Care for our Wounded Warriors and our Veterans, between $200/mo (oral, including opioids) but before more invasive, spinal procedures are considered ($5K - $30K/procedure). Injectable GCs have been used for over 50 years. Our targeted GCs are readily adaptable to pen-injectable form to treat multiple forms of localized pain such as forms of at-level SCI pain, lower back pain, or phantom limb pain. Our pen-injectable non-opiate concept is suitable for military use since it (i) is resistant to substance abuse, (ii) does not interfere with cognitive or cardiovascular function, and (iii) can be self- or buddy-administered.

Shown: Novo Nordisk insulin pens



Next Steps: De-risk Translational Development

Next steps. Our next immediate steps in translational development focus on efficacy and safety, to: (1) define a Therapeutic Profile, by test article (FA-rhNGF, FA-rhBDNF or their combination); route and site, where we do not expect a linear dose-response[14,15]; and dose/-schedule in established models of chronic pain; and (2) safety, as defined by GLP 7- & 28-day toxicology/pharmacokinetic studies and Quantitative Whole Body Analysis studies.

Future studies: IND, clinical studies & FDA approval. We view formulation for a pen-injectable device as feasible, since self-injectable pens are widely-used, particularly in Europe, and since protein-drug conjugates are readily lyophilized. The Manzanita team, expert consultants and vendors have a track record of (i) completing studies to budget and to Milestone (DARPA, DOD, SBIR/NCI); (ii) conducting translational studies through to Investigational New Drug (IND) application; and (iii) obtaining FDA approval for novel analgesic agents.

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