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Targeted Glucocorticoids: Preliminary Data Support Development for Spinal Cord Injury Pain

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Challenges of Treating Spinal Cord Injury Pain

The Department of Veterans Affairs Veterans Health Administration (VA) cares for ~15% of all individuals with spinal cord injury (SCI) pain in the U.S.[1] Veterans represents 20% of the 200,000 persons in the U.S. with SCI at an estimated economic cost of $9.7 billion.[2] The incidence of pain following spinal cord injury (SCI) varies widely, but averages around 65%.[3] About one-third of those with SCI report having severe pain.

Spinal cord injury is difficult to treat, often resistant to systemic glucocorticoids, analgesics, NSAIDs and even opiates.[4] Most agents given to treat SCI pain act centrally, resulting in cognitive impairment as well as CNS and dose-limiting toxicities. It is not definitively known whether SCI pain involves spinal and peripheral pathways, or both; or whether SCI pain is nociceptive or sensory, or both.[5]

We propose to target glucocorticoids (GCs) to the dorsal horn of the spinal cord, to the periphery and to the CNS. Our vision is a self-administered, non-opiate injectable product that is rapid acting and field-deployable, yet results in minimal cognitive impairment.

Glucocorticoids are potent inhibitors of pain because they act on multiple molecular targets including the key pro-inflammatory cytokines IL-1β, IL-6, calcitonin-related peptide (CGRP); the anti-inflammatory IL-10; prostaglandins; and cyclo-oxgenases (COX)-1 and -2.[7] The same broad activity results in dose-limiting toxicities when GCs are administered systemically. The effectiveness of epidural GCs to treat sciatica was first reported in the 1920's.[6] However, GCs localized to the dorsal horn can be 60%-80% effective for sciatica and neuropathic pain.[8,9]

Our drug of choice is the potent GC fluocinolone acetonide (FA). Our technology "targets" SCI pain by linking drug payload to a neurotrophin, a naturally-occurring protein of the nervous system, either Nerve Growth Factor (NGF) or brain-derived neurotrophic factor (BDNF). Both NGF and BDNF were studied as therapeutic agents in clinical trials (Genentech and Amgen, respectively), *ie* safety and dosing limits are known.

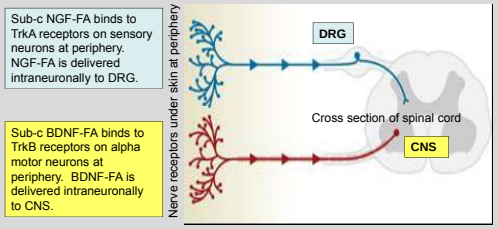

Figure 1. Differential Distribution.[15]

NGF (BDNF) is selective for TrkA (TrkB) receptors expressed on distal ends of sensory (alpha motor) neurons. When injected into any area of the affected dermatome, neurotrophins will be delivered to a central site, 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 of alpha motor neurons. The BNDF-TrkB complex is moved via retrograde axonal transport to the neuronal cell body, in the spinal cord, where it degrades.

We hypothesize that SCI pain may be modulated by targeted, intraneuronal delivery of GCs to the DRG and spinal cord. We will investigate and compare the effectiveness *in vivo* of peripheral injection of (1) FA to DRG (FA-NGF, selective for sensory neurons), or (2) spinal cord (FA-BDNF selective for alpha motor neurons), and/or (3) a combined dose.

Goal: Use Potent Payload & Localized Microdoses to Improve Therapeutic Index

Fluocinolone acetonide was selected as drug payload because it is ~20X stronger than methylprednisolone and ~4X stronger than dexamethasone. By increasing the potency of the payload, preliminary studies suggest that we reduce the absolute amount of NGF to levels known to be well tolerated in the clinic. We selected the rat model for neuropathic pain, zoster-induced allodynia, because it is clinically relevant, is focal, but involves minimal damage to retrograde axonal transport (Figure 2).[10]

In preliminary studies our approach required 2,500x or 7,000x less conventional drug (celecoxib and gabapentin, respectively) than our targeted study material to achieve similar levels of pain relief.

Preclinical Data: Targeted Glucocorticoids

Figure 2 shows the rapid and sustained activity *in vivo* of the conjugate, the potent glucocorticoid fluocinolone acetonide (FA) conjugated to NGF. NGF-FA given intramuscularly (im) exploits NGF-TrkA mediated retrograde axonal transport to deliver drug from the periphery to the nerve cell body. The conjugate was effective after 4 im injections 1x-daily of 0.34μg FA per dose (5μg NGF per dose equivalent).

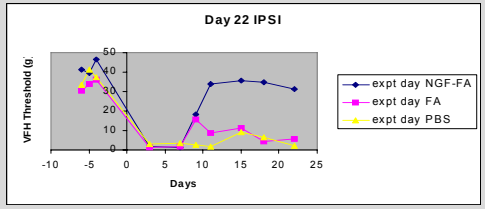

Figure 2. NDC (NGF-Fluocinolone Acetonide Conjugate) is Active in a Model for Post-Herpetic Neuralgia[10]

Procedure and Results. 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. Paw withdrawal threshold dropped from ~ 35 g to 1-3 g (VFH). NGF-FA 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.)

NGF = Nerve Growth Factor
FA = Fluocinolone acetonide, an approved topically administered glucocorticoid
PBS = Phosphate Buffered Saline
VFH Threshold = Von Frey Hair Threshold, a quantitative mechanical measure of pressure applied to the footpad

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. As shown below in **Figure 3,** unconjugated FA showed almost no activity compared to the conjugated GC variant. **Figure 4** gives controls of unbound NGF at doses at and above amounts that proved effective.

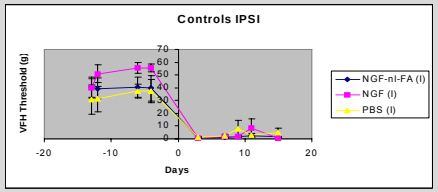

Figure 3. Controls (Ipsilateral = ipsi)

◆ NGF-nl-FA = Nerve Growth factor mixed with FA, not conjugated

■ NGF = Nerve Growth Factor alone, no FA or linker (native protein)

▲ PBS = Phosphate Buffered Saline

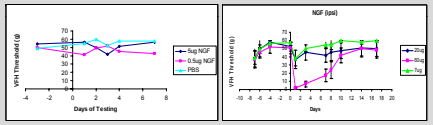

Figure 4. NGF Unbound Well-Tolerated At Doses Consistent with Clinical Reports

Figure 4 shows two studies of unbound NGF. On the left, NGF unbound was administered in the model at levels similar to results described in Figure 2 and also 10X that used in Figure 2. In the right panel, single injections of still greater amounts, at 7μg, 20μg and 60μg showed a transient, dose-dependent effect similar to results from clinical studies (Genentech[12,13]).

In Vitro Proof

Using Alexa Fluor 488 as a proxy for a small drug molecule, we developed an NGF-488 conjugate to confirm TrkA binding activity. First we confirmed TrkA binding activity of NGF-488 in mass neuronal culture. Then in **Figure 5** we showed TrkA-mediated delivery of NGF-488 to the neuronal cell body in the compartmented culture system developed by RB Campenot.[14,15]

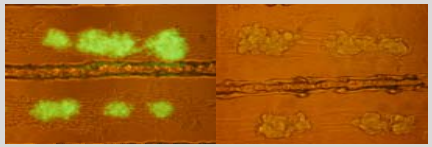

Figure 5. TrkA-Mediated Delivery of NDC to Neurons[11]

Figure 5 shows the cell bodies of neurons in the central compartment of the compartmented culture system. The left panel shows ***fluorescent*** NGF in cell bodies after 10 ng/ml ***f***NGF was applied to distal ends of sympathetic neurons in a compartmented culture system. In control studies in the right panel, receptor-mediated activity was confirmed by adding 1000 ng/ml unlabeled NGF to 100 ng/ml ***f***NGF. The addition of excess unlabeled NGF will compete the labeled NGF (NGF-488) off the TrkA receptors. The company observed that 1000 ng/ml unlabeled NGF abolishes transport of labeled NGF shows that the transport of NGF-488 requires receptor binding.

Following studies that showed analgesic effect in several preclinical models, NGF was shown to be non-immunogenic and well-tolerated (0.1 μg/kg) and even suggested a reduction in pain in Phase 2 and Phase 3 trials (Genentech).[13,14]

Conclusion

Our development plan plots a shortest time possible to clinical proof by (1) frontloading technical and clinical risk, (2) selecting clinically relevant pain models, and (3) careful choice of a first disease target. Our clinical trial design incorporates established endpoints and a relatively short time-to-endpoint read-out.

A secondary aim of our development strategy is to convert our interdisciplinary, unique capability to protocols that may be utilized by non-experts. All materials for manufacturing are commercially available in the U.S. Since we view dosing as our main clinical risk, we include extensive dose range-finding studies in our preclinical studes before proceeding to our Phase 1a study. De-risking our project upfront will allow us to access a larger pool of industry and private capital to leverage military funding support.

In conclusion, we showed that a novel targeted glucocorticoid in a clinically relevant model exhibited rapid onset of action and sustained anti-allodynic activity following 4 1x-daily im injections at very small absolute amounts of GC, 0.34μg per dose. By contrast, in the same model, gabapentin and celecoxib given by oral gavage required 7,000x and 2,500x more total drug, respectively, to reach an analgesic effect that was not sustained.

Our preliminary findings suggest that dosing would remain below establihsed clinical safety for unbound neurotrophin. Although our findings are novel, they are consistent with other reports that pain is be modulated at the genetic level.[16,17]

Because BDNF is approx. 60% homologous to NGF, and because delivery to the CNS via TrkB-mediated activity is known, we hypothesize that a BDNF-FA conjugate may also prove effective. It is possible that one or both conjugates may prove effective in treating SCI pain, depending on the nature and location of the actual nerve injury.

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 SCI pain, localized chronic pain or phantom limb pain. Our pen-injectable non-opiate concept is suitable for military use since it (1) is resistant to substance abuse, (2) does not interfere with cognitive function, (3) is field-deployable, and (4) can be self- or buddy-administered.

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