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**14. ABSTRACT**

We aimed to develop novel, non-addictive, treatments for acute soft tissue and skeletal injuries, such as seen in battlefield trauma, without the problems associated with opioid drugs. We targeted the delivery of small, membrane impermeable, sodium channel blockers through large pore TRP channels, such as TRPV1. These channels act as the innate "trigger" of nociceptive and inflammatory pain and are open at the site of acute injury and during the phases of inflammatory pain that follow. During the funded period we completed *in vitro* pharmacological screening of 46 new chemical entities (including exclusion of compounds with activity on cardiomyocytes) and identified 5 that showed promise relative to the reference compound, QX-314. In vivo studies in acute inflammatory and post-surgical pain models identified BW031 as the most promising lead for future therapeutic exploration as treatment for battle wound and surgical injures.

**15. SUBJECT TERMS**

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The overall goal of our project was to develop a new approach to the management of acute pain caused by battle wounds that can be applied quickly and safely and by untrained personnel, and which does not have the problems associated with morphine or similar opioid drugs. To do this we conducted a formal *in vitro* and *in vivo* drug screening program to identify novel cationic sodium channel blockers capable of selectively blocking nociceptors for prolonged periods, with the aim to identify safe and effective drug candidates, suitable for further development and ultimately progression into human clinical trials.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

pain, traumatic injury, battlefield wounds, analgesics, sodium-channel blockers, lidocaine, opioids, morphine, non-addictive analgesics, pain control

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

**What were the major goals of the project?**
*List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

The major goals of the project were:
1. Identify novel compounds (New Chemical Entities, NCEs) that are potent intracellular sodium channel blockers capable of permeation through TRPV1 channels.

2. Use NCE hits for in vivo testing that show no extracellular CVS or CNS activity and that permeate through TRPV1 in DRG neurons.

3. Identify 3 best NCEs ranked by analgesic profile and lack of motor impairment.

4. Determine analgesic $IC_{50}$, and assess biodistribution, tolerance and toxicity of candidate NCEs.

**What was accomplished under these goals?**

*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

1) **Identify novel compounds (New Chemical Entities, NCEs) that are potent intracellular sodium channel blockers capable of permeation through TRPV1 channels.**

As proposed, we worked with Dr. Jinbo Lee, a highly experienced medicinal chemist, to design and synthesize a series of novel permanently charged (cationic) sodium channel inhibitors and then tested the compounds for potency in inhibiting human Nav1.7 sodium channels, using a cell line that we made in which human Nav1.7 channels are stably expressed in human embryonic kidney (HEK) cells. The design, synthesis, and testing of the compounds were done in an iterative manner, using the results of each round to guide the next round of design of the compounds. Our goal was to design novel compounds with enhanced potency for blocking human Nav1.7 channels compared to QX-314 (N-ethyl-lidocaine), an already-existing tool compound with which we started the project.

We were highly successful in meeting the goals of the Aim. We ended up identifying 14 compounds with enhanced inhibition compared to QX-314. The best of these compounds were then carried through the other Aims of the overall project and shown to have activity in *in vivo* analgesic tests.

As a result of this work, the technology and compounds have been licensed by a newly-formed company Nocion, who will carry on the work to commercialize the discoveries and do clinical testing, with the goal of developing new treatments for pain, itch, and cough.

The work supported during the grant period, together with the work done to obtain preliminary results for the proposal and the work done while waiting for the grant period to begin, resulted in the synthesis and testing of a total of 34 compounds comprising a variety of chemical scaffolds. Of these, 18 were identified as most promising hits in several waves during the grant period. The iterative process of functional testing and designing of new compounds was highly successful, and the most potent compounds were three code named BW-031, BW-044 and BW-045. The design of the new compounds was done in close collaboration with Dr. Jinbo Lee, the consulting medicinal chemist. Dr. Lee also designed the synthetic pathways for the novel compounds and worked with the chemists at the chemical company doing the synthesis to address problems that arose during the synthesis. Dr. Lee also reviewed the quality control data for purity before the compounds were shipped.

A key part of the test of potency of Nav1.7 inhibition by the compounds was testing for the property of use-dependent inhibition, whereby the degree of inhibition increases with increasing cycling of the sodium channel through cycles of opening and closing. This is a desirable property because it means that activity of a nerve fiber will be blocked more effectively as the nerve fiber is firing more, as when it is transmitting pain generating signals. All the more potent compounds displayed strong use-dependence, which was most prominent for the current lead candidate, BW-045.

In addition to testing the novel compounds for potency and use-dependence of block of Nav1.7 channels when applied inside the cell expressing the Nav1.7 channels, an additional criterion for selecting the optimal compound was lack of inhibition when the compound is applied extracellularly. This is a desirable property for our strategy because our goal was to develop compounds that only block pain-sensing neurons expressing large-pore channels like TRPV1, TRPA1, and P2X receptors, which mediate pain signaling by responding to agents released during tissue damage like ATP and protons or agents released during inflammation like endocannabinoids. By only inhibiting neurons after entering through TRPV1, TRPA1, or P2X channels, we selectively inhibit only pain-sensing neurons without producing paralysis (from block of motor neurons) or general numbness (from block of non-painful touch) or producing undesirable side-effects from block of autonomic neurons. Most but not all the 14 compounds that blocked Nav1.7 channels better than QX-314 with intracellular application, also had the desirable property of not blocking effectively with extracellular application. This condition was met by best compounds,BW-031, BW-044 and BW-045. Those compounds that did not meet this test were not carried forward into the next series of tests described in the subsequent Aims.

The design of multiple novel compounds with increased potency over QX-314 for inhibiting human Nav1.7 channels when present intracellularly but not extracellularly successfully met the proposed goals of this Aim.

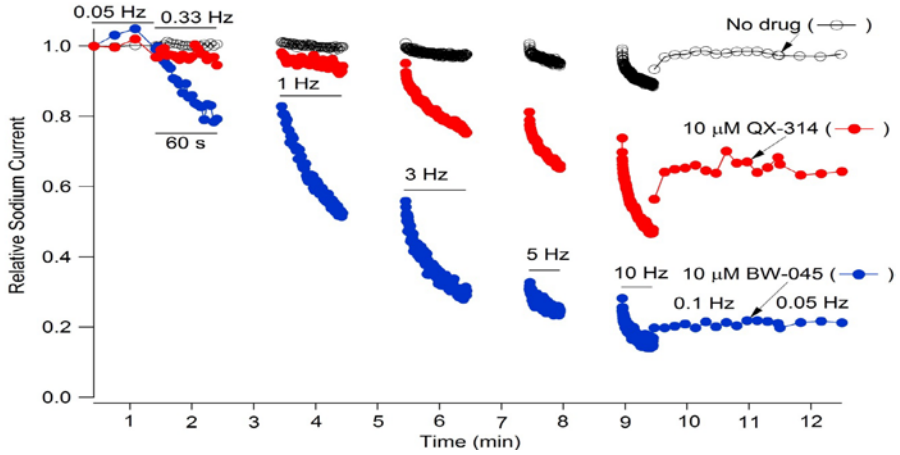



**Figure 1**. Time-course of voltage-dependent sodium current recorded from Nav1.7-expressing HEK cells treated with 10 micromolar intracellular QX-314 (red) or 10 micromolar BW-045, one of the novel charged compounds (blue) designed, synthesized, and tested in Aim 1. Compounds were applied in the recording pipette dialyzing the cell with intracellular solution. Sodium current was evoked by 20-msec steps from -100 mV to -20 mV, delivered at an increasing series of frequencies. BW-045 produces more inhibition than QX-314. Also, while QX-314 requires stimulation frequencies of 3 Hz or greater for substantial use-dependent block, BW-045 produces use-dependent block at frequencies as low as 0.33 Hz. Note the lack of recovery from inhibition by BW-045 when the stimulation was slowed from 10 Hz to 0.05 Hz, as if the cationic compound is trapped within the channels when channels are closed.

2) **Obtain NCEs in volumes sufficient for in vivo testing (5g of each NCE) that show no extracellular CVS or CNS activity and that permeate through TRPV1 in DRG neurons.** Having identified new chemical entities (NCEs) which blocked voltage-gated $Na_v1.7$ sodium channels in human embryonic kidney (HEK) cells intracellularly with greater potency as compared to BW001 (QX-314), we then proceeded to further test these compounds *in vitro*. Using a formal screening funnel to identify hits with all the desired characteristics. Specifically, we wanted to confirm that the potent NCEs also permeated into and blocked Nav currents in TRPV1 expressing mouse primary sensory dorsal root ganglion (DRG) neurons. Additionally, we wanted to measure the potency and selectivity of the NCEs for extracellular block of $Na_v1.7$ and $Na_v1.5$ in HEK cells *in vitro* via high throughput screening. Whereas the $Na_v1.7$ channel is mainly expressed in somatosensory neurons and was our primary molecular target, the $Na_v1.5$ channel is primarily expressed in cardiomyocytes and its block could be predictive of potential cardiotoxicity. Finally, we also wanted to directly measure the effect of the NCEs on human induced pluripotent stem cell derived cardiomyocytes *in vitro* as a more direct measurement of potential cardiotoxicity.

The NCEs selected for additional *in vitro* screening included BW001 – our reference compound, as well as BW004, BW005, BW031, BW035 and BW041. All of the latter NCEs at 10 μM exhibited more potent use-dependent internal block of $Na_v1.7$ channels in HEK cells as compared to BW001 and also blocked no more than 20% of the $Na_v1.7$ current in HEK cells in a use-dependent manner when applied extracellularly, suggesting they would be more likely to be selective for neurons expressing large-pore channels which allow for intracellular entry of the charged NCEs. These five NCEs and the reference compound BW001 thus proceeded to the next step of our screening pipeline.

These NCEs were then measured for extracellular block of Nav1.5 and Nav1.7 sodium channels in HEK cells via a thallium flux assay. The thallium assay is a high throughput method of measuring sodium and/or potassium channel function and has recently been used to screen for sodium channel subtype selective blockers (Du et al., 2015). The two channels Nav1.5 and Nav1.7 sodium were selected as the focus for the screen since the $Na_v1.5$ channel is a key channel expressed in cardiac myocytes, where it drives the depolarization of the cardiac action potential (Abriel, 2010), whereas the $Na_v1.7$ channel is primarily expressed in primary somatosensory neurons, in particular in nociceptors, where it is critical for pain sensation (Dib-Hajj et al., 2007). After screening the six compounds described above, we found that none of them exhibited significant extracellular block of either the $Na_v1.5$ or $Na_v1.7$ sodium channels (Table 1), suggesting their activity is selective for internal Nav channel block. There was no apparent subtype selectivity for any of the compounds tested at high millimolar concentrations, suggesting they are not selective for $Na_v1.7$.

After characterizing the external block of $Na_v1.5$ and $Na_v1.7$, we proceeded to test whether these compounds could permeate into dorsal root ganglion (DRG) neurons and block endogenous Nav currents in them, which are comprised of $Na_v1.7$, $Na_v1.8$ and other sodium channels (Dib-Hajj et al., 2007). Adult mouse DRGs were dissected, dissociated and treated

with capsaicin together with each NCE separately. Capsaicin is an agonist of large-pore that allows the entry of QX-314 (BW001) and other large molecules (Binshtok et al., 2007). After pretreatment, the NCEs were washed off and the sodium current from TRPV1 expressing neurons was measured by whole cell patch clamp electrophysiology. We found that the sodium currents in DRG neurons treated with the NCEs were considerably smaller than those from neurons treated with capsaicin alone (Table 1), suggesting that the NCEs did, indeed permeate into TRPV1 expressing DRG neurons and blocked endogenous $Na_v$ currents.

In order to get a preliminary measure of potential NCE toxicity, we recorded the effect of the NCEs on the activity of human IPSC-derived cardiomyocytes *in vitro*. While our proposed means of administering the NCEs is a topical treatment via a subcutaneous injection, gel or aerosol, it may be possible for NCEs to enter the bloodstream. Thus, it is important to ensure that the compounds would not result in cardiotoxicity in that scenario, a potential concern given that they can block cardiac $Na_v1.5$ sodium channels at high concentrations. Five out of 6 NCEs had minimal effect on cardiomyocyte activity at doses up to 1mM (Table 1), which is similar to the dose of NCEs that was used to inject subcutaneously and should be considered the upper limit of systemic exposure. One of the NCEs, BW041, blocked cardiomyocyte activity almost completely and was thus eliminated from further testing.

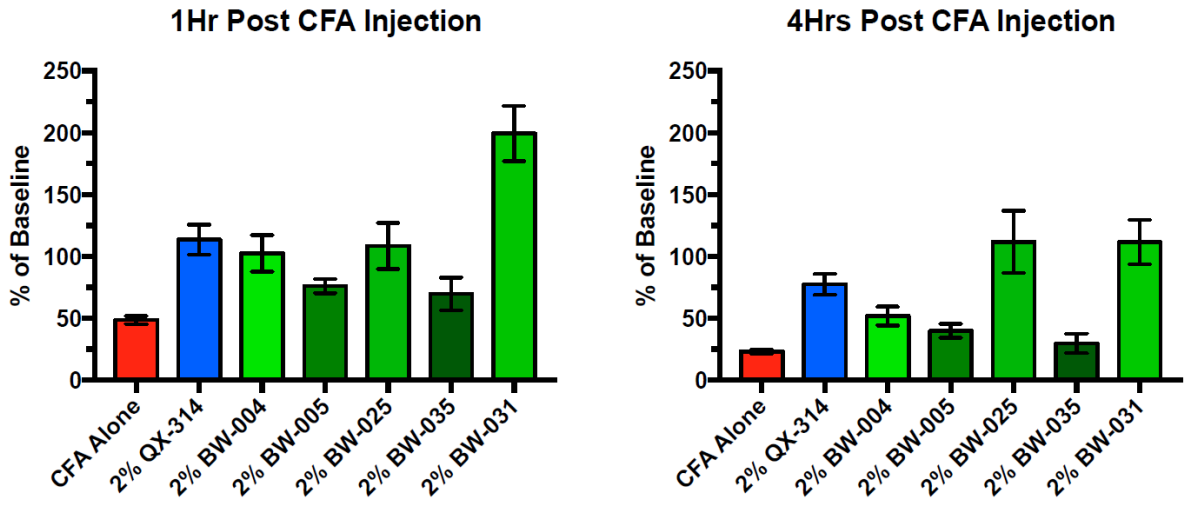
The results of our *in vitro* testing validate our experimental design and screening strategy, given the relatively high rate of compounds that satisfied the requirements we set out. Based on these results, we identified a total of 4 NCEs – BW004, BW005, BW031 and BW035 - that have a satisfactory pharmacological profile and were suited for further *in vivo* testing. These compounds were then tested *in vivo* in animal models of acute pain and their pharmacokinetic profile was also measured *in vivo*.

**Table 1** below summarizes the *in vitro* NCE screening data. A brief description of each assay is given at the top, with criteria for advancing compounds into further studies in the middle and data from the various assays at the bottom. The NCEs satisfying the filter criteria are highlighted in green, and those failing them in red. For a more detailed description of each experiment, see the materials and methods.

| Color Legend: | $Na_v1.7$ internal use-dependent block | $Na_v1.7$ external use-dependent block | Extracellular block of $Na_v1.5/Na_v1.7$ | | DRG neuron permeation | Block of cardiomyocyte activity | Compounds advancing to *in vivo* studies |
|---|---|---|---|---|---|---|---|
| **Advancing compounds** | Use-dependent block of $Na_v1.7$ in HEK cells by 10 µM NCE applied internally. Measured by whole cell patch clamp electrophysiology. | Use-dependent block of $Na_v1.7$ by extracellullarly applied NCEs at 10X the estimated IC50 for internal block. Measured by whole cell patch clamp electrophysiology. | Extracellular block of $Na_v1.5$ / $Na_v1.7$ in HEK cells by 100 µM NCEs. Measured by the thallium assay. | | NCE block of Nav currents in TRPV1+ DRG neurons using patch clamp electrophysiology after pretreatment with 100 µM compound + capsaicin. | Assess extracellular block of pacing in human iPSC derived cardiomyocytes in vitro using calcium imaging | |
| **Failed compounds** | **Filter: More block by compound as compared to QX-314** | **Filter: < 20% block** | **Filter: <15% block.** | | **Filter: >40% sodium current block.** | **Filter: <20% block of cardiomyocyte activity (calcium wave amplitude) at 1 mM compound** | |
| **Top NCEs:** | **$Na_v1.7$ internal block (%)** | **$Na_v1.7$ external block (%)** | **$Na_v1.5$ block (%)** | **$Na_v1.7$ block (%)** | **$Na_v$ current block** | **Cardiomyocyte activity block** | |
| **BW-001** | 37% | 0% | 3% | 0% | 62% | 4% | Yes (reference) |
| **BW-004** | 79% | 2% | 0% | 0% | 86% | 1% | Yes |
| **BW-005** | 74% | 14% | 0% | 0% | 82% | 1% | Maybe (backup) |
| **BW-031** | 65% | 15% | 0% | 8% | 73% | 7% | Yes |
| **BW-035** | 48% | 2% | 0% | 13% | 54% | 0% | Maybe (backup) |
| **BW-041** | 44% | 9% | 0% | 1% | 60% | 98% | No |

3) **Identify 3 best NCEs ranked by analgesic profile and lack of motor impairment.** Following from the *in vitro* screening undertaken in Aims 1 and 2 we advanced 5 compounds (BW004, BW005, BW025, BW031 and BW035) plus QX314 (BW001) to *in vivo* assessment for efficacy. The initial screening assay we used was a model of acute inflammation; intra-plantar CFA. This is a commonly used model of peripheral inflammatory pain provoked by the inflammatory response resulting from a combination of mycobacterial wall extract and lipids, and is characterized by local swelling, erythema and prolonged thermal and mechanical hyperalgesia. Eight week old male Sprague Dawley rats (Charles River) were housed in the animal facility 2 weeks prior to testing and housed 3 per cage on a 12h light/dark cycle with food (standard chow) and water ad libitum. Three days prior to testing a compound (only one compound was tested per experiment at one concentration – 2% w/v) and thermal hypersensitivity was measured using the Hargreaves radiant heat test. The sensitivity to heat was measured and 24h afterwards, CFA was co-administered with test compound by intra-plantar injection into the plantar region of one hind paw. Heat sensitivity was measured at 1h and 4h post-injection by an observer blind to treatment.

A]




B]

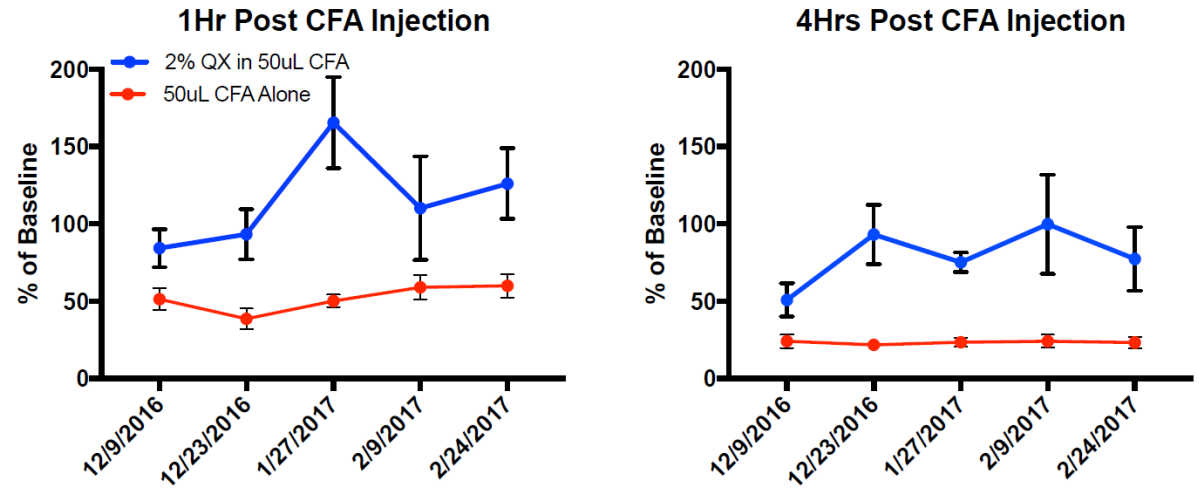



**Figure 2.** A shows that at 1h post-injection of CFA, several compounds had comparable efficacy to QX314 (BW001) but that BW031 was clearly the most efficacious. At 4h post-injection, BW025 and BW031 were more efficacious than BW001 indicating a longer duration of action in this model of acute inflammation. In Figure 2 B it can be seen than CFA caused consistent heat hyperalgesia and the comparator compound BW001 (QX314) consistently reduced heat hyperalgesia across experiments (each date corresponds to a different compound tested, aligned with the compounds in panel A).

Based on our findings in the CFA model we tested BW001 (QX314) and BW031 in a model of acute post-surgical pain; the plantar incision model. The threshold of sensitivity to mechanical stimulation using a mechanical von Frey device was determined the day prior to surgery. On the day of surgery rats were anesthetized and a short incision made in the plantar surface of one hind paw under aseptic conditions. 24h after the incision was made rats were tested for mechanical allodynia. Animals were then dosed with saline, BW001 (QX314; 2%)

or BW031 (2%) and at various time points post-injection, rats were tested for mechanical sensitivity to von Frey stimulation.

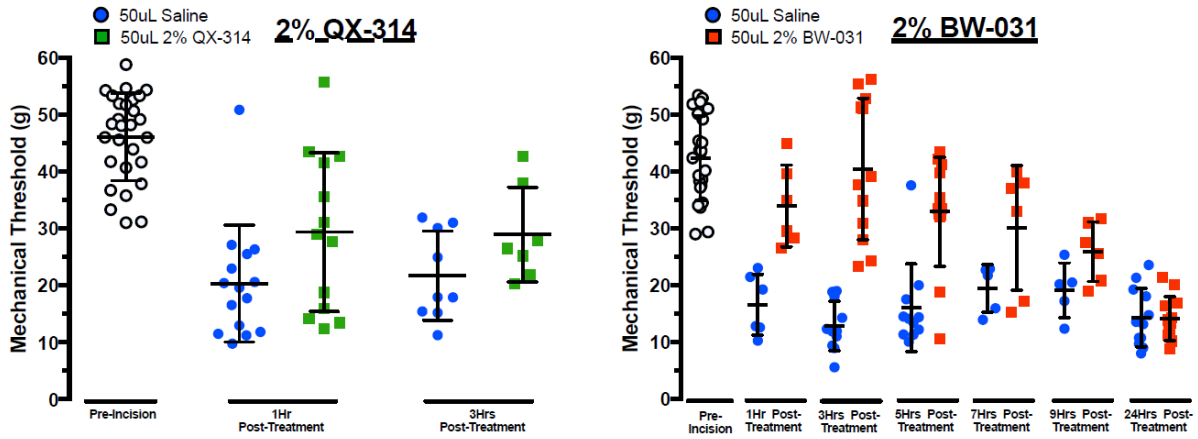



**Figure 3.** BW031 showed efficacy to reduce mechanical hypersensitivity up to 7h post-injection and at 9h post-injection.

4) **Determine analgesic IC50, and assess biodistribution, tolerance and toxicity of candidate NCEs.**
Following demonstration of the longer duration of action of BW031 relative to BW001 (QX314) in the incisional model, we tested lower concentrations of BW031 (1 and 0.5% w/v).

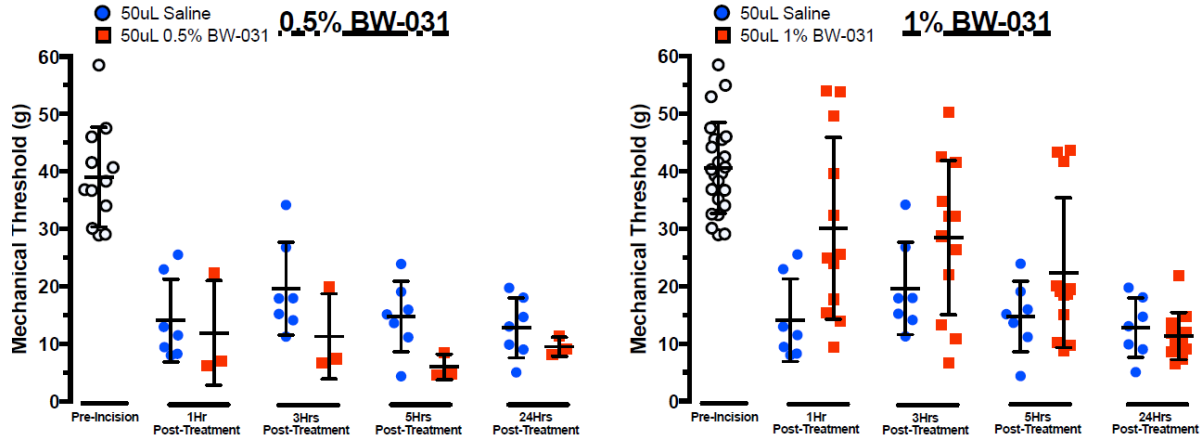



**Figure 4.** BW031 (1%) showed efficacy and a prolonged duration of action in the incisional model but had no effect at 0.5% w/v.

We established an analytical assay for detection of BW001 (QX314) in plasma and used this to measure levels of compound in plasma following intra-plantar and intra-venous administration. Under anesthesia, rats (n=3) were implanted with a cannula into the jugular vein and the following day administered 2% BW001 (QX314) into the plantar region of one hind paw. Blood samples were taken at 5 min, 15min, 30min, 60 min, 90min, 120min and 240min post-intra-plantar injection. In a separate rat from the ones treated with BW001 intra-plantar, BW001 was administered intravenously in the same concentration and volume as that given intra-plantar to determine what a 100% exposure at that dose and volume would be. It was postulated that one of the metabolic products of BW001 (QX314) could be lidocaine and so that was also measured in the samples.

**Table 2**

| File Name | QX314 (nanoM) | Lidocaine (nanoM) |
|---|---|---|
| Rat 01 Baseline | ND | ND |
| Rat 01 5 min | ND | ND |
| Rat 01 15 min | ND | ND |
| Rat 01 30 min | ND | ND |
| Rat 01 60 min | ND | ND |
| Rat 01 90 min | ND | ND |
| Rat 01 120 min | ND | ND |
| Rat 01 240 min | ND | ND |
| Rat 02 Baseline | ND | ND |
| Rat 02 5 min | ND | ND |
| Rat 02 15 min | ND | ND |
| Rat 02 30 min | ND | ND |
| Rat 02 60 min | ND | ND |
| Rat 02 90 min | ND | ND |
| Rat 02 120 min | ND | ND |
| Rat 02 240 min | ND | ND |
| Rat 03 Baseline | ND | ND |
| Rat 03 5 min | ND | ND |
| Rat 03 15 min | ND | ND |
| Rat 03 30 min | ND | ND |
| Rat 03 60 min | ND | ND |
| Rat 03 90 min | ND | ND |
| Rat 03 120 min | ND | ND |
| Rat 03 240 min | ND | ND |
| | | |
| ND = below the LLOQ | | |
| LLOQ (Lowest Limit of Quantification) = 20nM | | |

As can be seen in Table 2, levels of BW001 (QX314) and lidocaine were below the limit of quantification (20nM). Based on these data there is predicted to be at least a 1000 fold separation between the concentration of QX-314 that reaches the systemic circulation and that which inhibits Nav1.5 channels in the heart since LLoQ of QX-314 was 20nM and 100µM QX314 does not inhibit the channel (in-house data). Similarly, lidocaine has an IC50 of approx. 200µM at Nav1.5 channels (data from Cerep.com).

When the bolus of BW001 (QX-314) was injected intravenously, QX-314 was detectable in the first 5 min sample (24µM) Table 3. The concentration injected was 30mM so the maximum possible to retrieve was 57µM indicating that it was rapidly eliminated.

**Table 3**

| | QX314 (uM) |
|---|---|
| Rat 04 Baseline | BLOQ |
| Rat 04 5 min | 24.60 |
| Rat 04 15 min | 1.422 |
| Rat 04 30 min | 0.3628 |
| Rat 04 60 min | 1.911 |
| Rat 04 90 min | BLOQ |
| Rat 04 120 min | BLOQ |
| Rat 04 240 min | 1.634 |

The pharmacokinetic experiments were repeated for our candidate BW031 and samples are currently being analyzed. We are also evaluating the histology of the plantar skin of the paw 24h post-injection of BW031 for evidence of any acute, local toxicity.

**What opportunities for training and professional development has the project provided?**
*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

| |
|---|
| Nothing to Report. |

**How were the results disseminated to communities of interest?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

| |
|---|
| A poster was presented at the conference organized by the DoD in August, 2018. A manuscript is in preparation for submission to a peer reviewed journal. |

**What do you plan to do during the next reporting period to accomplish the goals?**
*If this is the final report, state "Nothing to Report."*

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

| |
|---|
| Nothing to Report. |

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

**What was the impact on the development of the principal discipline(s) of the project?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

| |
|---|
| We have used a series of efficacy and safety drug discovery screens (screening funnel) to identify non-opioid activating, new chemical entities that show promise as potential clinical candidates for treatment of pain, such as the immediate pain of wounded soldiers while they are being evacuated to a hospital setting. The screens have allowed us to identify a lead compound (BW031), which if shown to be safe in phase 1 and effective in phase 2 clinical studies, may be able to provide prolonged block of acute pain caused by battle wounds, without the problems associated with opioid compounds. |

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

In addition to blocking "pain signals," the population of nerves that we are silencing are also expressed by lung sensory neurons, where they detect pain, cough and other forms of lung irritation. With this in mind, we have tested the lead candidates identified through our screening funnel in a Guinea pig cough model. We found that BW031 can silence chemical lung irritation and could therefore be a potential candidate for treatment of, or prophylaxis for, chemical warfare agents and other lung irritants that could be encountered in the battlefield.

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Our end goal is to develop a new therapy capable of relieving the acute pain generated by battlefield injury. This same strategy could also be applied to the management of postoperative pain for elective or emergency surgery. To arrive at one or more of these clinical end goals, we will partner with industry and/or private investment firms to ensure that this technology is transferred into the clinical development pipeline for subsequent clinical trials.

**What was the impact on society beyond science and technology?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

**Changes in approach and reasons for change**
*Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

Nothing to report.

**Actual or anticipated problems or delays and actions or plans to resolve them**
*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

Nothing to Report.

**Changes that had a significant impact on expenditures**
*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

Nothing to Report.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
*Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

**Significant changes in use or care of human subjects**

Nothing to Report.

The IACUC protocol came to an end as there is a 3 year cycle and this supported the efforts of the grant.

**Significant changes in use of biohazards and/or select agents**

Nothing to Report.

**6. PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  **Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

| |
|---|
| Nothing to Report. |

**Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

| |
|---|
| Nothing to Report. |

**Other publications, conference papers, and presentations**. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

| |
|---|
| Nothing to Report. |

- **Website(s) or other Internet site(s)**
  *List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

| |
|---|
| Nothing to Report. |

- **Technologies or techniques**
  *Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.*

| |
|---|
| Nothing to Report. |

- **Inventions, patent applications, and/or licenses**
  *Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

| |
|---|
| Nothing to Report. |

- **Other Products**
  *Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

| |
|---|
| Nothing to Report. |

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."*

| | |
|---|---|
| *Name:* | *Clifford Woolf, MB, BCh, PhD* |
| *Project Role:* | *Co-PI* |
| *Researcher Identifier (e.g. ORCID ID):* | *0000-0002-6636-3897* |
| *Nearest person month worked:* | *1* |
| *Contribution to Project:* | *Dr. Woolf assisted in the determination of identity of new chemical entities and supervised the establishment of in vitro and in vivo assays.* |
| *Name:* | *Bruce Bean, PhD* |
| *Project Role:* | *Co-PI* |
| *Researcher Identifier (e.g. ORCID ID):* | *0000-0002-5093-3576* |
| *Nearest person month worked:* | *1* |
| *Contribution to Project:* | *Dr. Bean helped identify of new chemical entities and supervised the in vitro testing of new compounds.* |
| *Name:* | *Nick Andrews, PhD* |
| *Project Role:* | *PD* |
| *Researcher Identifier (e.g. ORCID ID):* | *0000-0001-9966-5093* |
| *Nearest person month worked:* | *12* |
| *Contribution to Project:* | *Dr. Andrews oversaw in vitro and in vivo assay development and testing, coordinated data recording and submission protocols, and prepared reports.* |

| | |
|---|---|
| *Name:* | *Benjamin Doyle, MS* |
| *Project Role:* | *Research Assistant* |
| *Researcher Identifier (e.g. ORCID ID):* | *0000-0002-9228-4121* |
| *Nearest person month worked:* | *12* |
| *Contribution to Project:* | *Mr. Doyle performed in vitro and in vivo assays.* |

| | |
|---|---|
| *Name:* | *Ivan Tochitsky, PhD* |
| *Project Role:* | *Research Fellow* |
| *Researcher Identifier (e.g. ORCID ID):* | *0000-0003-0650-9193* |
| *Nearest person month worked:* | *6* |
| *Contribution to Project:* | *Dr. Tochitsky developed in vitro assays and performed in vitro screening experiments.* |

| | |
|---|---|
| *Name:* | *Sooyeon Jo, PhD* |
| *Project Role:* | *Research Fellow* |
| *Researcher Identifier (e.g. ORCID ID):* | *0000-0001-5555-6514* |
| *Nearest person month worked:* | *9* |
| *Contribution to Project:* | *Dr. Jo developed in vitro assays and performed in vitro experiments.* |

| | |
|---|---|
| *Name:* | *Gui-Lan Yao, MD, PhD* |
| *Project Role:* | *Research Associate* |
| *Researcher Identifier (e.g. ORCID ID):* | *0000-0003-2940-9443* |
| *Nearest person month worked:* | *1* |
| *Contribution to Project:* | *Dr. Yao has supported the electrophysiology experiments by maintaining tissue culture equipment and supplies.* |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

A new grant from the American Academy of Otolaryngic Allergay began 9/15/18:
a) Title: Role of TRPV1+ sensory neurons in driving IgE production in allergic diseases
b) Principal Investigator: Clifford Woolf, MB, BCh, Ph.D.
c) Goals: the goal of this project is to begin to ask how sensory neurons act on B cells to influence their ability to produce IgE.
d) Specific aims/tasks 1) Using an in vitro co-culture system developed in our lab, ask whether sensory neurons influence IgE production via contact dependent or independent mechanisms (2) test specific action of neuropeptides on IgE production in vitro.
e) Start and end date: 9/15/18-9/14/19
f) Level of effort: 0.12 calendar months

**What other organizations were involved as partners?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*
*Provide the following information for each partnership:*
*Organization Name:*
*Location of Organization: (if foreign location list country)*
*Partner's contribution to the project (identify one or more)*

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

We received grants supporting *in vivo* drug development assays for charged sodium blockers from the Boston Biomedical Innovation Center (NIH funded) in the amount of $250,000, and a similar drug development grant from an anonymous foundation, also for $250,000. These grants are both in support of the parallel development of this technology for the treatment of cough by silencing nociceptors in the lung.

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:** For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

Nothing to Report.

**QUAD CHARTS:** If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

## 9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Nothing to Report.

# Development of Novel Local Analgesics for Management of Acute Injury Pain

Log Number: MR141339  Task Title: Final Report Quad Chart

Award Number: W81XWH-15-1-0480

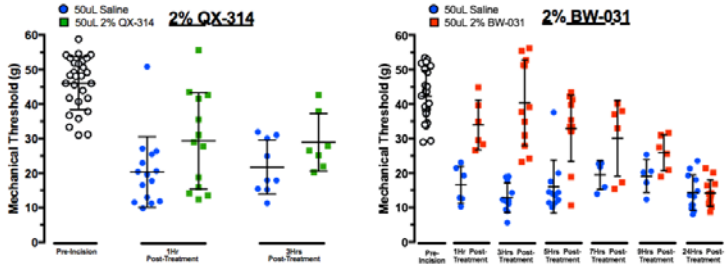
**PI:** CJ Woolf & B Bean  **Org:** Boston Children's Hospital / Harvard Medical School  **Award Amount:** $1,500,000

## Study/Product Aim(s)

- Synthesize and test compounds for intracellular but not extracellular Na-channel blocking activity
- Assess permeation through TRPV1 channels and test for CNS and CVS toxicity
- Demonstrate long lasting selective analgesic activity in inflammation and tissue injury models
- Study systemic redistribution and tolerability

## Approach

Our objective is to develop a local/regional analgesic by targeting small charged sodium channel blockers into nociceptor neurons through large pore TRP channels.




BW031 has superior duration of action and efficacy at a comparable concentration when compared with QX-314 in the rat plantar incision model pf post-surgical pain. BW-031 also more efficacious and long lasting in the CFA model of inflammation

**OUTCOME:** Favorable in vitro efficacy and lack of activity on cardiomyocites indicating good predicted safety, longer duration of action and greater efficacy in vivo, BW031 is our lead compound..

**OVERALL Accomplishment:** BW-031 identified as lead compound from 46 new chemical entities tested in vitro (selectivity/efficacy/safety). 5 NCEs were tested in vivo – BW-031 was the most efficacious and long lasting.

## Timeline and Cost

| Activities | CY 15 | 16 | 17 | 18 |
|---|---|---|---|---|
| Produce candidate charged compounds | | | | |
| Evaluate pore permeation and blocking activity | | | | |
| Test analgesic activity of leads in vivo | | | | |
| Biodistribution, safety and toxicity testing | | | | |
| **Estimated Budget ($K)** | **$500** | **$500** | **$500** | |

**Updated:** November 2018

**Goals/Milestones:**

- ☑ **CY15-17 Goal** – Produce set of charged sodium channel blocker new chemical entities (NCEs).
- ☑ **CY15-18 Goal** – Identify most potent intracellular sodium channel blockers capable of permeation through TRPV1.
- ☑ **CY15-18 Goal** – Determine permeation through TRPV1 in DRGs.
- ☑ **CY15-18 Goal** – Obtain NCEs in sufficient quantities for *in vivo* testing
- ☑ **CY16-18 Goal** – Test for NCEs without extracellular CNS/CVS activity.
- ☑ **CY16-18 Goal** – Evaluate/rank NCEs for analgesic activity, selectivity.
- ☑ **CY16-18 Goal** – Determine analgesic $IC_{50}$ for the selected NCEs.
- ☑ **CY16-18 Goal** – Evaluate local and systemic safety and tolerability

**Comments/Challenges/Issues/Concerns**

- No substantial challenges, issues or concerns
- Difference in actual vs. projected expenditure due to deferred costs

**Budget Expenditure to Date**

Projected Expenditure: $1,500,000

Actual Expenditure:  $1,500,000