Evaluation of $Na_V1.7$ Inhibitors for the Treatment of Ocular Pain

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SITEONE THERAPEUTICS

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♦ Purpose

Ocular pain, a symptom in various ocular pathologies, is a predominant reason for patients to visit an ophthalmologist or optometrist for evaluation. Ocular pain accompanying eye diseases can be triggered by inflammation and or direct peripheral or central neuronal injury. Currently, there is a significant unmet medical need for effective, safe and long lasting topical ophthalmic analgesics suitable for patient self-administration. SiteOne Therapeutics has developed a series of highly-selective small molecule inhibitors of a specific human voltage-gated sodium channel $Na_V1.7$, the isotype responsible for initiation of the pain signal. The objective of this study was to profile compounds from SiteOne library for initial ocular efficacy, safety, and duration of ocular tissue exposure.

♦ Methods

Mouse model of corneal anesthesia (from Wang *et al.* [2]). Following topical administration of a test article (10 µl), corneal sensitivity was tested at 5, 10, 15, 30 min, 1 hour, 2, 4 hours. At each time point, corneal sensitivity was evaluated using a Cochet–Bonnet esthesiometer (Luneau Ophtalmologie, Chartres, France), which consists of a retractable nylon monofilament with a diameter of 0.12 mm. At the shortest 0.5 cm length, a lack of response indicates complete anesthesia, whereas absence of response at the longest 6 cm length indicates partial block, and is indicative of a more analgesic and less anesthetic state.

Ocular pharmacokinetics (PK) and toxicity in rabbits. Topical ocular application of a Nav1.7 small molecule inhibitor was evaluated in New Zealand White rabbits as a standard ophthalmic model species using a modified McDonald-Shadduck scoring system[3]. Tolerability outcome measures included standard ocular endpoints detectable with slit-lamp photography, fluorescein staining, and clinical ophthalmic examinations were conducted during pre-screen baseline, daily on days 1-3 and twice daily on day 4 of the study. The scores range from 0 (no abnormalities found) to 4 (severe damage). Exposure and ocular tissue distribution were analyzed using plasma samples and collected ocular tissues from treated animals (n=4 eyes/time point) at baseline and 15, 30, 60, 120, 240, and 480 minutes post dose.

All experimental procedures were approved by an Institutional Animal Care and Use Committee in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and conducted at an AAALAC-accredited facility.

♦ Results

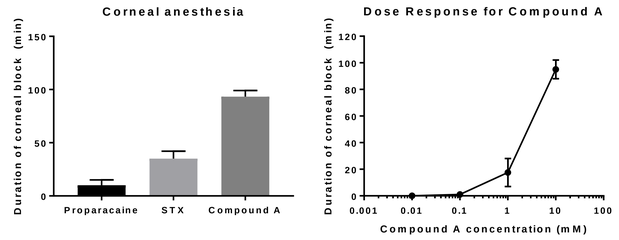


Figure 1. Corneal anesthesia with non-selective compounds, natural guanidinium toxins and local anesthetics. **(Left)** The duration of corneal anesthesia following single topical application of 15 mM proparacaine, 1 mM saxitoxin (STX) and 10 mM Compound A (non-selective Na_V inhibitor from SiteOne library) was assessed in a mouse model of corneal tactile sensitivity with Cochet-Bonnet esthesiometer at 0.5 cm length. Note: topical administration of STX and Compound A paralyzed the iris muscle leading to the loss of pupillary response. **(Right)** Dose-response relationship for Compound A. Error bars: standard deviation.

These results demonstrate that bis-guinadiunium compounds reach corneal nerve fibers after topical administration. However, off-target effect on the iris muscle prevented further development of these non-selective compounds. The absence of ocular pain in genetically null $Na_V1.7$ individuals along with the excessive ocular pain in patients with $Na_V1.7$ gain of function strongly suggest that $Na_V1.7$ is the major Na_V isotype responsible for pain signaling in the eye and that non-$Na_V1.7$ isotypes mediate ocular protective reflexes. Therefore, the effort of the project shifted to evaluation of $Na_V1.7$-selective compounds.

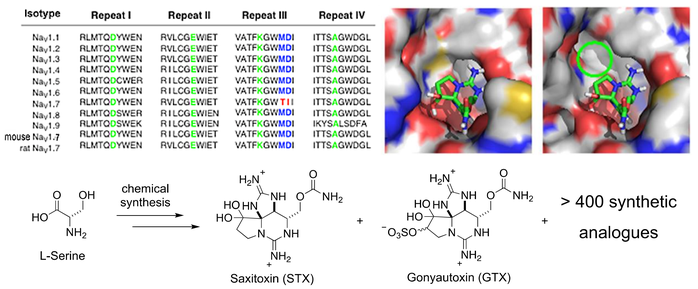
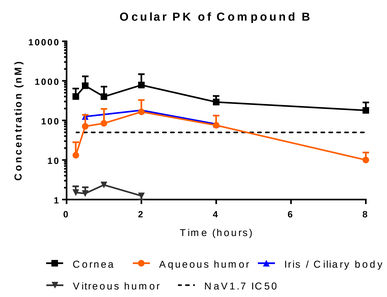
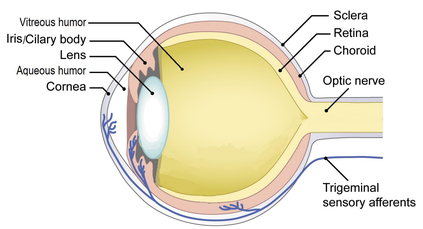


Figure 2. Discovery of potent and selective inhibitors of $Na_V1.7$ (Left). Primary sequence comparison of the pore loop regions for human Na_V isoforms reveals a two amino acid variation in repeat III that is unique to the $Na_V1.7$ subtype. Amino acids comprising the selectivity filter DEKA are highlighted in green. **(Right).** STX docked to $Na_V1.4$ (left) and the identical pose in hNaV1.7 (right) highlighting the unique structural differences at Site 1 (green circle). **(Bottom).** *De novo* synthesis of guanidinium toxin derivatives makes possible assessment of structure activity relationships at eight positions. Over the past 4 years, we have prepared a collection of ~400 bis-guanidinium derivatives bearing substituents at different positions. *Several of compounds in our library display sub 100 nM potency against $hNa_V1.7$ and over 1000-fold selectivity against all other hNa_V isotypes.* These results validate our strategy for selective ligand design, and indicate that compounds with >1000-fold selectivity for $hNa_V1.7$ vs all other hNa_V isotypes can be readily achieved. Our lead compounds are negative in in-vitro cytotoxicity and mutagenicity assays; they display excellent liver microsome stability, low protein binding and minimal activity against safety targets in-vitro.



4-day repeat dose ocular tolerability scores

	Days			
Ocular tissue examined	**1**	**2**	**3**	**4**
Conjunctiva	0	0	0	0
Cornea	0	0	0	0
Pupillary light response	0	0	0	0
Anterior chamber	0	0	0	0
Iris	0	0	0	0
Lens	0	0	0	0
Vitreous	0	0	0	0
Retina	0	0	0	0
Choroid	0	0	0	0



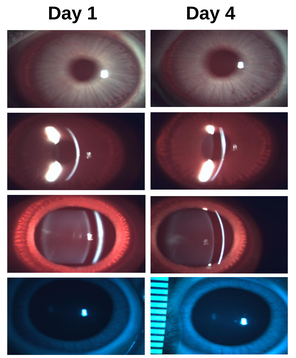


Figure 3. Ocular pharmacokinetics (PK), tolerability and toxicity of a $Na_V1.7$-selective compound. To determine whether therapeutically effective concentrations of a $Na_V1.7$ selective compound can be achieved in the eye by topical ocular administration, and to determine ocular tolerability of such administration, we conducted PK and tolerability studies in rabbits with Compound B (15 mg/ml). **(Top Left)** PK analysis from single dose administration of 1.5% Compound B. Ocular distribution was maintained above the Compound B's human $Na_V1.7$ IC50 in the iris and ciliary body as well as aqueous humor for at least 240 minutes, with cornea concentrations above the human $Na_V1.7$ IC50 for over 480 minutes. Vitreous test article concentrations were below the measured $Na_V1.7$ IC50 throughout the study, while retina and blood plasma concentrations were below detection limits. Error bars: standard deviation. **(Top Right)** No ocular anomalies, adverse general health effects, or adverse effects on body weight were observed during the four days of twice daily topical ophthalmic 1.5% Compound B dosing. The table summarizes ocular examinations on each day of the study. Scores range from 0 (no abnormalities) to 4 (severe toxicity). **(Bottom Left)** Schematic diagram showing ocular structures of interest and innervation by sensory trigeminal fibers that carry pain signal from the eye. **(Bottom Right)** Representative slit-lamp images from the first and last days of dosing. Note the absence of corneal erosions (fluorescein signal in the bottom panels).

Topical ophthalmic administration of 1.5% Compound B did not cause corneal surface abnormalities that are present after even a single administration of topical lidocaine or proparacaine, thereby demonstrating the comparative safety of our selective Na_V inhibitors relative to the standard of care. PK analysis from single dose administration of 1.5% Compound B showed that the concentration of drug remained above 200 nM for almost 8 hours, suggesting that greater than 80% block of $Na_V1.7$ is possible with a single topical application. Based on our PK-pharmacodynamics understanding, this level of block translates to strong analgesia.

♦ Conclusion

A selective $Na_V1.7$ small molecule inhibitor demonstrated initial tolerability and target ocular tissue test article distribution following topical dosing using rabbits as a standard ophthalmic model species. These findings highlight the potential to develop Nav1.7 inhibitors for use in the treatment of acute and chronic ocular pain and discomfort associated with dry eye syndrome, corneal abrasions, corneal infections, and post-operative pain following ocular surgical procedures.

♦ About SiteOne Therapeutics

SiteOne Therapeutics is an early-stage biotechnology company founded in 2010 by Stanford University researchers and a biotech entrepreneur-scientist. SiteOne Therapeutics is headquartered in Bozeman, Montana with a research laboratory in the South San Francisco, California. Since its inception, SiteOne has been dedicated to developing novel pain therapeutics to safely, effectively and efficiently treat acute and chronic pain without the limitations of existing pain therapies, such as NSAIDs or opioids. The company's therapeutic candidates are highly selective sodium ion channel 1.7 ($Na_V1.7$) inhibitors based on naturally occurring small molecules. Given the urgent need for new, non-opioid solutions for managing pain, SiteOne is focused on advancing its lead product candidates for multiple therapeutic applications. Results from this study demonstrate the promise of a $Na_V1.7$-selective inhibitor for the development of a safe, effective, long lasting topical ophthalmic analgesic.

More information about SiteOne Therapeutics can be found at https://www.siteonetherapeutics.com/.

♦ References

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(2) Wang L, Shankarappa SA, Tong R, Ciolino JB, Tsui JH, et al. (2013) Topical drug formulations for prolonged corneal anesthesia. Cornea 32: 1040–1045.
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♦ Disclosures

AD, HP, GM, JM: SiteOne employees
DY, JDB: SiteOne consultants

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