Identification of a selective voltage gated sodium channel blocker able to preferentially inhibit human dorsal root ganglia neurons sensitized by inflammatory agents

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Early Human Insights

Introduction

Safe and effective treatment of patients with chronic pain conditions remains an unmet medical need. Further compounding this problem, current therapeutic options are often associated with serious side effects. It is in this context that a major effort has been undertaken by pharmaceutical companies over the last few decades to discover and develop novel analgesic drugs. Unfortunately, efforts to develop new analgesics have been largely unsuccessful as most preclinical animal models, have failed to translate in clinical settings. In order to overcome the translational challenges, we have developed methodologies for enabling the large-scale utilization of human primary cells and tissues in drug discovery and clinical candidate selection. We now report on a novel potential therapeutic identified by relying on this new human-focused discovery paradigm.

Methods

Organs and tissues were perfused with AnaBios' proprietary solution to preserve tissue viability. Dorsal root ganglia (DRG) were enzymatically dissociated and seeded on collagen-coated glass coverslips. Voltage-gated sodium channel (VGSC) blockers were identified in a library screening campaign using a combination of high throughput imaging-based with electrical field stimulation-(EFS) assays and voltage clamp electrophysiology in HEK cells. The molecules that exhibited selectivity against VGSC were further tested in human DRG neurons using a combination of electrophysiology and calcium imaging. Cardiac tissues and isolated cardiomyocytes were used to assess drug selectivity by measuring inotropic risk in trabeculae and isolated myocyte contractility assays and pro-arrhythmic risk.

Results

ANB-504	Nav1.7 inact. (nM)	Nav1.7 tonic (nM)	Nav1.8 inact. (nM)	Selectivity over Nav1.5	Selectivity over Nav1.2
	700	>30,000	800	>10x	>10x

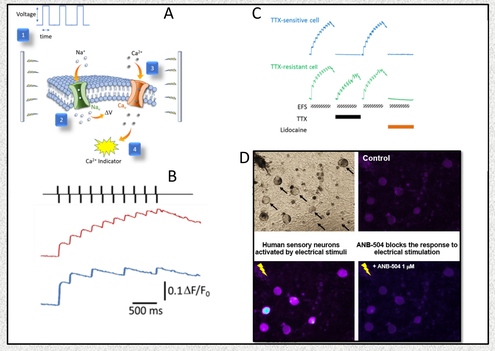

Figure 1. ANB-504 inhibition of hDRG Activation. A: EFS-based imaging assay. **B**: Stimulation-induced calcium transients. **C**: under the EFS stimulation used the responses are VGSC-dependent. **D**: Inhibition of EFS-induced responses by ANB-504.

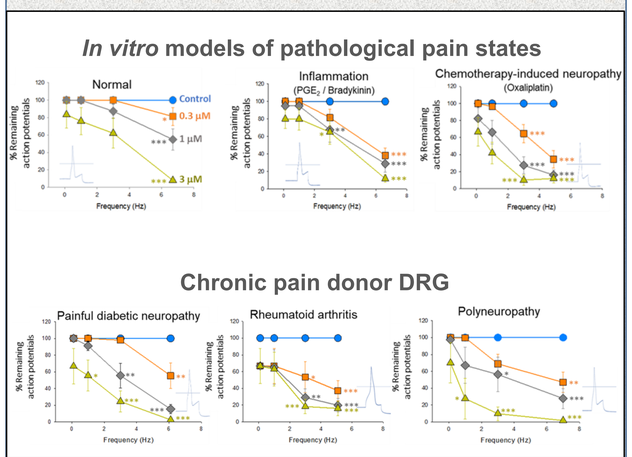

Figure 2. ANB-504 Inhibits the Activation of Human Nociceptors in Pathological Conditions.
Human DRG neurons in culture were used in I-clamp recordings, while cells were stimulated to induce trains of 120 consecutive action potentials at the indicated frequencies. Inhibition of the action potentials was observed in the presence of increasing concentrations of ANB-504 (0.3µM, orange; 1µM, gray; 3µM, green). For all of the concentrations tested, it is also apparent the use-dependence of the inhibitory effect of ANB-504: the increase in the cell firing frequency resulted in more pronounced inhibition of the action potentials.

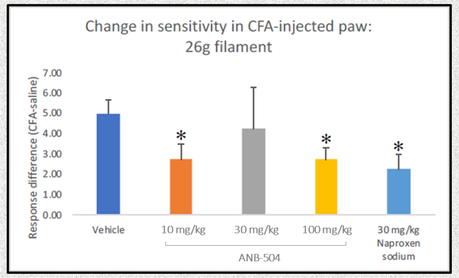

Figure 3. Effect of ANB-504 in the CFA Inflammatory Pain Model in Rat.
The lowest dose tested already produced an analgesic effect comparable to that of the positive control Naproxen.

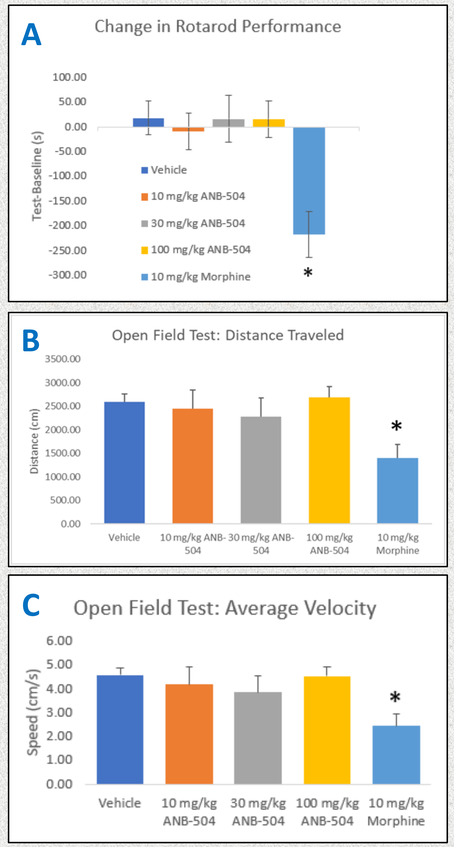

Figure 4. ANB-504 Does Not Exhibit CNS-related Side Effects in Rat.
A. Rotarod test performance assessed 1 hr after drug administration.
B. Open field distance travelled measured 1 hr after drug administration.
C. Open field average velocity measured 1 hr after drug administration.

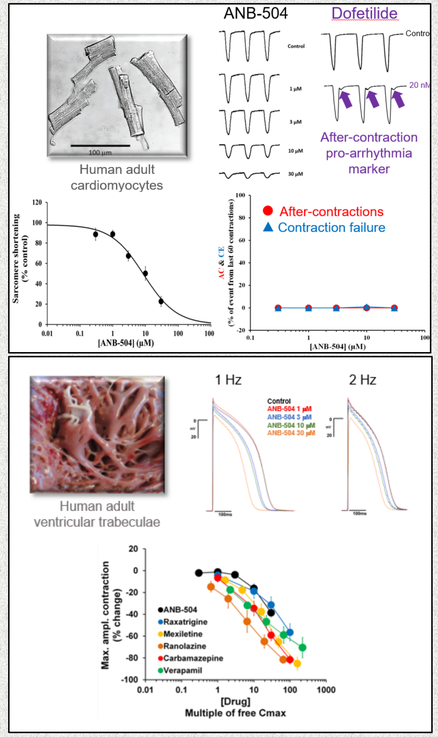

Figure 5. ANB-504 Does Not Affect Human Cardiac Tissue Function.
A. Cardiac contraction transients recorded from human ventricular cardiomyocytes.
B. Cardiac action potentials and contractions recorded from human ventricular trabeculae.

Summary

- We have identified ANB-504 as a novel use-dependent blocker of VGSC $Na_v1.7$ and $Na_v1.8$.
- ANB-504 inhibits the activation of human sensory neurons in pathological states.
- ANB-504 is also active (starting at 10 mg/kg) in a rat inflammatory pain model.
- ANB-504 does not exhibit CNS-related side effects in rat at doses as high as 100 mg/kg.
- ANB-504 had undetectable effect on the human cardiac action potential and exhibits excellent safety margin with respect to cardiac contractility risk.
- ANB-504 has excellent non-clinical ADMET profile and its development is underway