Ex vivo human models of inflammatory and neuropathic pain states for enabling translational research and drug discovery

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Introduction

The development of new pain therapeutics has been hampered by the lack of reliable preclinical models. To address this challenge we have established a novel preclinical discovery strategy, which relies on the ex vivo interrogation of dorsal root sensory neurons isolated from human organ donors. Here, we present a model for studying the efficacy of new compounds to treat different types of pain. By adding proinflammatory or neuropathic agents to culture conditions, we investigated how the physiological properties of cells are altered in our pain models.

Results

Inflammation Increases the Percentage of TTX-resistant Cells

Figure 3. Fluo-8-AM Calcium Imaging of hDRGs

A

B

C

Figure 4. Inflammation Changes the Functional Phenotype of hDRGs

A

B

C

Figure 5. Distinct NaV1.7 and NaV1.8 Blocker Efficacy in Models of Pain

A

B

C

Figure 6. NMDA receptor antagonists by the NaV1.7-potentiation Model: PBR541-971441. A. Control condition; n=10. B. 100 nM PBR1, and 50 nM PBR2 for 72 hours; n=5. C. 50 μM Oxaliplatin; n=10 Oxaliplatin; n=10 Oxaliplatin.

Figure 7. NaV1.7 and NaV1.8 Blocker Efficacy in Models of Pain

A

B

C

Figure 8. NMDA receptor antagonists by the NaV1.7-potentiation Model: PBR541-971441. A. Control condition; n=10. B. 100 nM PBR1, and 50 nM PBR2 for 72 hours; n=5. C. 50 μM Oxaliplatin; n=10 Oxaliplatin; n=10 Oxaliplatin.

Figure 9. NMDA receptor antagonists by the NaV1.7-potentiation Model: PBR541-971441. A. Control condition; n=10. B. 100 nM PBR1, and 50 nM PBR2 for 72 hours; n=5. C. 50 μM Oxaliplatin; n=10 Oxaliplatin; n=10 Oxaliplatin.

Methods

Cell isolation & culture. Human dorsal root ganglia (hDRGs) were collected within 2 hours post-mortem and transferred to AnaBios using proprietary cold preservation solution. Cells were enzymatically dissociated, plated on PDL-coated glass coverslips, and cultured in DMEM-F12 in the presence of 10 ng/mL NGF and 10 ng/mL GDNF, at 37°C with 5% CO2.

Fluorescence calcium imaging. hDRG neurons were loaded with the calcium sensor Fluo-8-AM and imaged at 100 Hz with a popEDC SMCDS camera (Pico) mounted on an inverted microscope.

Electric field stimulation (EFS). A pair of carbon fiber rods was used as stimulating electrodes; trains of biphasic pulses with 10 ms duration and a frequency of 5 Hz were used to elicit calcium transients. Stimulus intensity was set at 2.5 V/cm to 15 V/cm.

Electrophysiology. Individual hDRG neurons were stimulated at frequencies of 0.1, 1, 3 and 10 Hz with trains of 120 depolarization pulses (20 ms in duration) delivered at 1500 Hz of rheobase. hDRGs were treated with 500 nM Prostaglandin E2 (PG_E2) and 50 nM bradykinin (BK) for 72 hours to model inflammation. To model chemotherapeutics-induced neuropathy, hDRGs were treated with 50 μM Oxaliplatin, or 100 nM Paclitaxel for 24 hours.

Conclusions

1. We successfully established human ex vivo models for inflammation and chemotherapy-induced neuropathy.
2. Our Ca2+ imaging approach enables the high-throughput characterization of pain-producing changes in functional properties of large numbers of hDRG neurons.
3. Our electrophysiological approach yields detailed single cell dose and frequency response relations of lead compounds.
4. The ability to model pain in human neurons is a powerful tool to predict the therapeutic efficacy of lead compounds under specific pain conditions.