



Models of Pain

MD Biosciences offers established pre-clinical models for evaluating potential therapies for Nociceptive, Neuropathic and Inflammatory Pain.

Neuropathic Pain:

- CCI Sciatic Nerve Ligation (Bennet & Zie Model)
- Spinal Nerve Ligaton (Chung Model)
- Taxol-induced Neuropathy
- STZ-Diabetic Neuropathy

Inflammatory Pain:

- Carrageenan-induced
- CFA-induced Inflammatory Pain

Post-operative Pain:

- Post-incisional pain in rats
- Post-incisional pain in pigs

Arthritic Pain:

- Adjuvant-induced Arthritis & Arthritic Pain

Nociceptive:

- Tail Flick Test
- Visceral Pain (acetic acid writhing test)
- Capsaicin

To speak to a scientist about evaluating a compound in a model of pain, email info-us@mdbiosciences.com or log on to www.mdbiosciences.com

FINDING BREAKTHROUGH THERAPIES IN THE TREATMENT OF CHRONIC PAIN

Pain is associated with the threat or presence of tissue damage and protects the body by warning us to avoid potentially harmful situations. We sense pain through the activation of sensory neurons. The "first-order" sensory neuron resides in the dorsal root ganglion (DRG). Axons from the neurons within these ganglia innervate our skin, organs, and bones. These fibers terminate either in specialized receptors that sense vibration, mechanical forces, heat, etc., or they terminate as free nerve endings that are exposed to the chemical environment of the tissue.

Nociceptors are specialized sensory neurons that preferentially respond to noxious stimuli. Nociceptive or physiological pain refers to the type of pain that we feel on a day-to-day basis. It is directly associated with a noxious stimulus, such as bending our joints too far, and helps us put limits on ourselves to avoid damage. This information is mediated by both small unmyelinated fibers and myelinated fibers, and provides us with an awareness of the environment. Nociceptive pain is pharmacologically treatable.

When pain continues beyond the original insult, it is generally associated with the healing process and reminds us to establish limits which allow our bodies to repair themselves. Under certain conditions, pain can continue long after the initial stimulus or occur even in the absence of any obvious damage. This phenomenon is referred to as chronic pain, a condition that affects millions worldwide.

The causes of neuropathic pain are mechanical nerve injury (either to periphery nerve or to the spinal cord) as results of tumor compression, trauma, ischemia, inflammation, diabetes and chemotherapy. It is a condition that develops after the original injury and is manifested by both constant (spontaneous) pain and an abnormal response to sensory stimuli (evoked activity) that is interpreted out of proportion to the intensity of the stimulus (that is, greatly exaggerated).

Depending on the type and cause of pain,

it may be treated with conventional analgesics/anti-inflammatory drugs such non-steroidal anti-inflammatory drugs (NSAIDs) or acetaminophen including ibuprofen or naproxen. More intense pain may require stronger treatment like opioid therapy; morphine, the prototypical opiate, is still commonly used to relieve severe pain. In addition, medications such as tricyclic antidepressants and anticonvulsants, initially approved for other conditions, have shown some efficacy in treating chronic neuropathic pain.

Despite many advances in the treatment of pain, some patients continue to struggle to find effective relief. Chronic pain, especially when neuropathic in origin, can become difficult to manage with long-term conventional therapy.

One challenge that arises in the search for an effective pain therapy is inconsistency in patient responsiveness to a given treatment, even among those who present similar symptoms. For example, medications used to treat chronic neuropathic pain typically show no more than a 40% to 60% success rate in providing clinically relevant relief to patients, and complete relief is often achieved in a much smaller proportion [1]. The incidence of negative side effects such as constipation, dizziness, and sedation may also limit treatment options. Additionally, patients may develop tolerance to certain medications after long-term use. Tolerance is a common problem among the some 6 million patients who are on prolonged opioid therapy for severe chronic pain. Because of the risk of abuse, many doctors are reluctant to prescribe high doses of opioids, leaving some patients with inadequate relief.

Ideally, pain therapy would eliminate nociceptive signals without affecting cognitive, motor, or other sensory functions. Unfortunately, none of the currently available treatments meet these criteria. For example, lidocaine, the most commonly used local anesthetic, interferes with neuronal signaling by diffusing into cells and blocking sodium channels from the inside. Although this mechanism of action is very effective in attenuating pain, it

does not allow discrimination between cell types. As a result, anti-nociceptive effects are accompanied by a paralysis, numbness, and disruption of autonomic input.

As basic scientists continue to make discoveries regarding the contribution of particular ion channels to sensory function, more selective targeting of pain signaling will be possible. A recently published study evaluated a novel strategy that may bring us closer to this goal. Researchers combined the administration of capsaicin, the active ingredient in hot peppers, and a lidocaine derivative called QX-314. Capsaicin acts at TRPV1 receptors, which are uniquely expressed in nociceptive neurons. Although QX-314 retains the anesthetic property of lidocaine, it is distinct in its inability to pass through the cell membrane under normal conditions. When capsaicin binds to TRPV1 receptors, a pore large enough for QX-314 to pass through is opened. Co-administration of capsaicin with QX-314 thereby allows the drug to selectively enter and inhibit nociceptors.

In this study, the effect of combined capsaicin and QX-314 treatment on neuronal excitability was first evaluated *in vitro* using cultured sensory neurons. While the application of QX-314 alone had little effect unless injected directly into cells, co-application with capsaicin achieved a nearly complete blockage of evoked sodium current, reflecting a substantial decrease in nociceptor excitability. This effect was observed only in TRPV1-expressing neurons, confirming that the drug's anesthetic actions are dependent on entrance into the cell through TRPV1 receptors.

The researchers then tested whether the selective effects would be maintained *in vivo*. In both heat and mechanical nociceptive tests, injection of capsaicin and QX-314 in combination into the hind paw or sciatic nerve of rats dramatically decreased sensitivity to noxious stimuli. As expected, QX-314 had no significant effect on nociceptive thresholds when injected alone. Furthermore, in contrast to lidocaine injection, the combined treatment resulted in successful maintenance of motor and tactile sensory function. This important difference demonstrates the value of this method in selectively targeting pain transmission without disrupting other functions [2].

This is the first study to exploit a channel expressed by a distinct group of neurons to deliver a drug exclusively to those cells. Building on this exciting new approach of using combination therapy to selectively target nociceptive function may eventually lead to the development of improved pain treatment for a variety of conditions wherein maintenance of cognitive, motor, sympathetic and tactile sensory function is favored. Importantly, this novel technique also holds promise for breakthroughs in the treatment of chronic pain.

References:

1. Markman JD and Dworkin RH. (2006) *J Pain*, 7(15): S38-47.
2. Binshtok AM, et al. (2007) *Nature*, 449(7162): 607-10.

MD Biosciences offers several in vivo models for the study of nociceptive, inflammatory and chronic pain. Custom design models and in vitro assays are also available.

Understanding How a Compound Affects Cell Adhesion and Migration

The recruitment and adhesion of leukocytes to areas of inflammation is a highly regulated process. Selectin-carbohydrate interactions slow down circulating leukocytes. Chemokines activate the appropriate cells through chemokine receptors on the tethered leukocytes. Finally, leukocyte integrins interact with components of the extracellular matrix and cell adhesion molecules on endothelial cells to form a tight bond. By understanding how a compound affects the adhesion process, one can predict the specific interactions being disrupted or enhanced. MD Biosciences offers the following *in vitro* assays for the study of cell adhesion and migration.

- Leukocyte migration assays. Fluorescently labeled cells (neutrophils, T-cells or monocytes) are incubated on a permeable cell culture insert above a chemoattractant. The number of cells migrating through the insert towards the signal are quantified by fluorescence intensity.
- Endothelial cell adhesion assay. Fluorescently labeled leukocytes are incubated on a monolayer of TNF- α stimulated endothelial cells. After a wash to remove the unbound cells, the bound leukocytes are quantified by fluorescence intensity.
- Integrin-mediated adhesion. Fluorescently labeled leukocytes are incubated in plates coated with various integrin ligands such as collagen I, collagen IV, fibronectin, laminin, vitronectin, ICAM-1, ICAM-2, VCAM-1 and MAdCAM-1. After a wash to remove the unbound cells, the bound leukocytes are quantified by fluorescence intensity.

To speak to a scientist about cell adhesion and migration *in vitro* assays, email info-us@mdbiosciences.com or log on to www.mdbiosciences.com