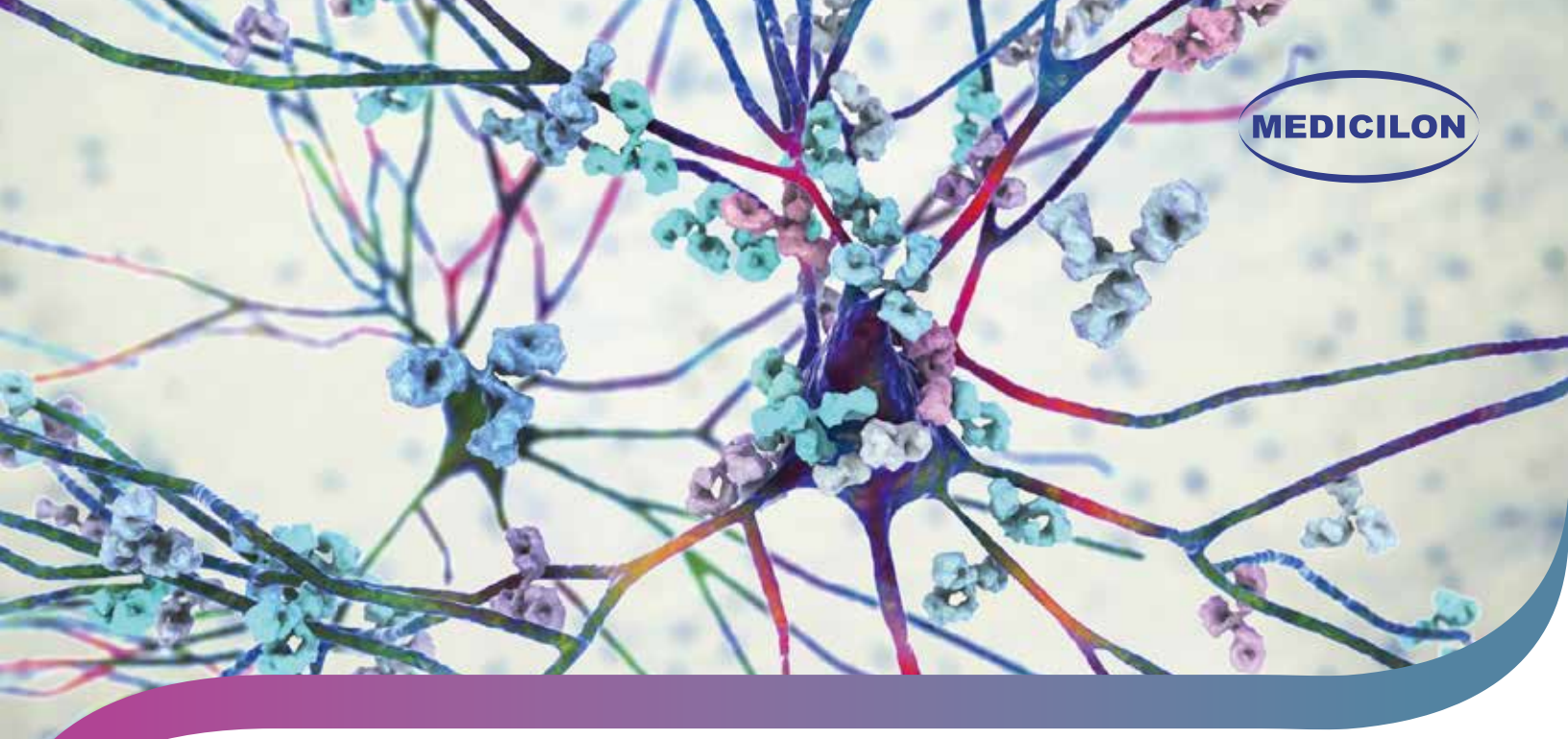


Medicilon Preclinical Nociceptive Pain Models

Pain is a sensation. IASP definition of pain (1979): “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” Revised IASP definition of pain (2020): “An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage.” Pain, both acute and chronic, remains a significant health problem despite tremendous progress in understanding of its basic mechanisms. Throughout ages man has used many different remedies for pain relief. However, there are still some pain symptoms can’t be effectively alleviated, and because of different degrees of analgesic drug adverse reactions or efficacy intensity and duration is not ideal, the prevention and treatment of pain is still not up to the ideal degree. Therefore, scientific researchers continue to invest a lot of energy and financial resources, hoping to develop new drugs with stronger analgesic effect, longer duration and lower side effects, for the benefit of mankind.

Physical pain can be acute and chronic, inflammatory and neurological, central and peripheral, etc. Therefore, various analgesics with different mechanisms of action and different types of analgesia need to be evaluated by corresponding animal models before clinical trials. When animals experience pain during an experimental protocol, a cascade of physiological, hormonal, biochemical, and behavioral alterations is triggered that’s simile the same way of human reaction. Therefore, we can choose the corresponding pain animal model to evaluate the new analgesic drugs.

A number of animal models have been developed, reflecting observations that pain phenotypes are mediated by distinct mechanisms. Animal models should be based on:

- understanding the clinical disease presentation and pathology;
- behavioral measures should be used that assess issues particular to that disease.

Animal models of pain are designed to mimic distinct clinical diseases to better evaluate underlying mechanisms and potential treatments. Outcome measures are designed to measure multiple parts of the pain experience including reflexive hyperalgesia measures, sensory and affective dimensions of pain and impact of pain on function and quality of life.

Currently, Medicilon possess different types of pain including acute pain, inflammatory pain, and neuropathic pain. We have developed many common methods used for inducing each of the pain phenotypes related to clinical pain syndromes, as well as the main behavioral tests for assessing pain in each model. We have developed a variety of clinically relevant pain models that have been validated to effectively test new therapeutic candidates.

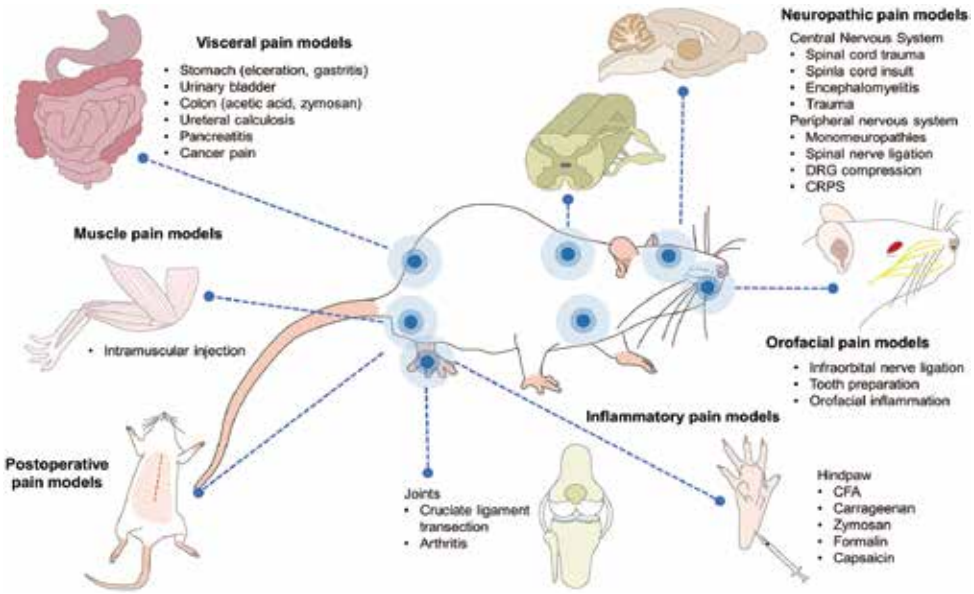


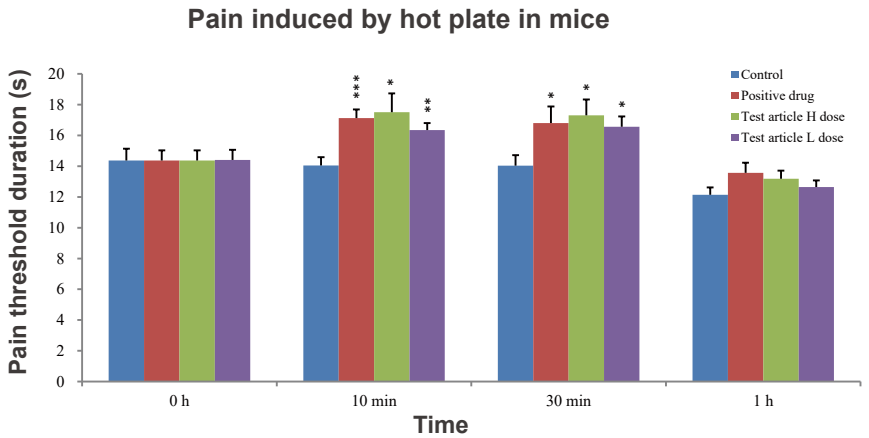
Figure 1. Pain models in rodents[1]

Hot-Plate Test

The hot plate test is a widely used experimental method to assess nociception in rodent models. The hot plate test involves placing the rodent on an enclosed hot plate and measuring the time it takes for them to respond (e.g. vocalization, paw lick, or paw raising). The time between placement of the mice on the hot-plate and the occurrence of either a hind paw lick or a jump off the surface was recorded as the hot-plate latency.

The advantages of this test are that it is objective, quantifiable, can be administered repeatedly without causing inflammation, and assesses supraspinally-organized responses to a noxious stimulus.

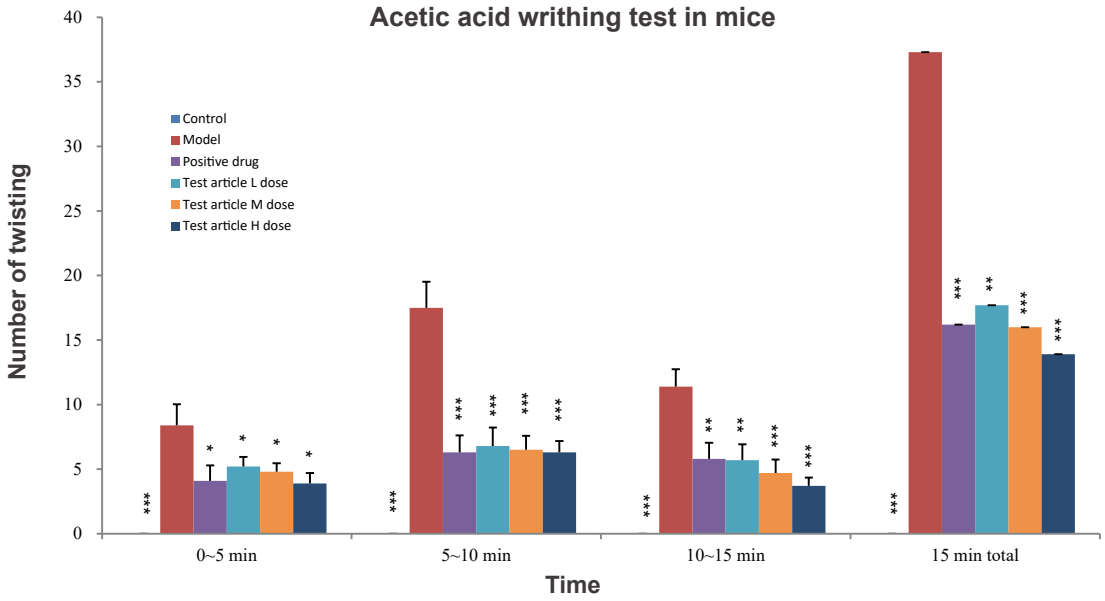
Medicilon Case: Hot-Plate test in mice



Acetic Acid Induced Writhing Test

Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principles like phenylquinone, acetic acid in mice. The method described by Koster et al. (1959) was used for the evaluation of analgesic activity in mice which evaluated the analgesic effect by using the acetic acid-induced writhing.

Medicilon Case: Acetic acid writhing test



Formalin-Induced Pain Model

The formalin model is widely used for evaluating the effects of analgesic compounds in laboratory animals. The formalin test in rodent is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs.

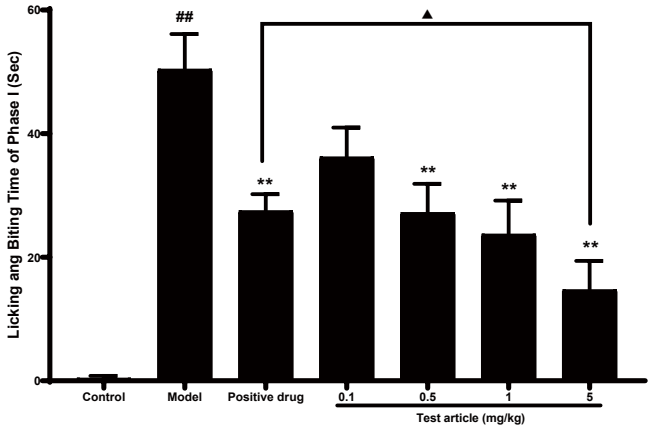
Injection of formalin into the hind paw induces a biphasic pain response. The effect of formalin is expressed by hindlimb licking and shaking that occur principally in two phases.

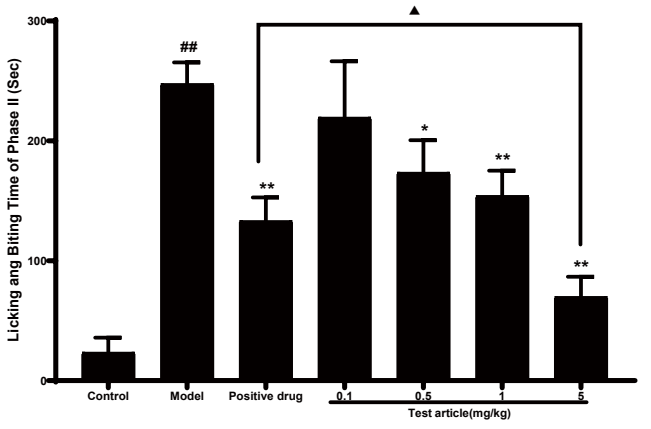
- Phase I: result from direct activation of primary afferent sensory neurons, an acute response to the formalin injection and occurs almost immediately after injection and lasting for between 5 to 10 minutes. Phase I pain mainly reflects neuropathic pain, and non-steroidal anti-inflammatory drugs have no effect on Phase I pain response;

- Phase II: reflect the combined effects of afferent input and central sensitization in the dorsal horn, lasts from approximately 20 to 40 minutes after injection is caused by a central sensitization activity. Both neuropathic pain and inflammatory pain are involved in Phase II pain, so both neuropathic analgesics and non-steroidal anti-inflammatory drugs have all analgesic effects on Phase II pain response.

The formalin model test has some advantages over other models, formalin injection was used as adequate stimulus and continuously induced nociceptive behaviors rather than transient ones in unrestrained animals, thus the precise onset and duration of analgesics can be assessed, also can distinguish between central and inflammatory analgesics.

Medicilon Case: Formalin induced pain model (phase I & phase II)

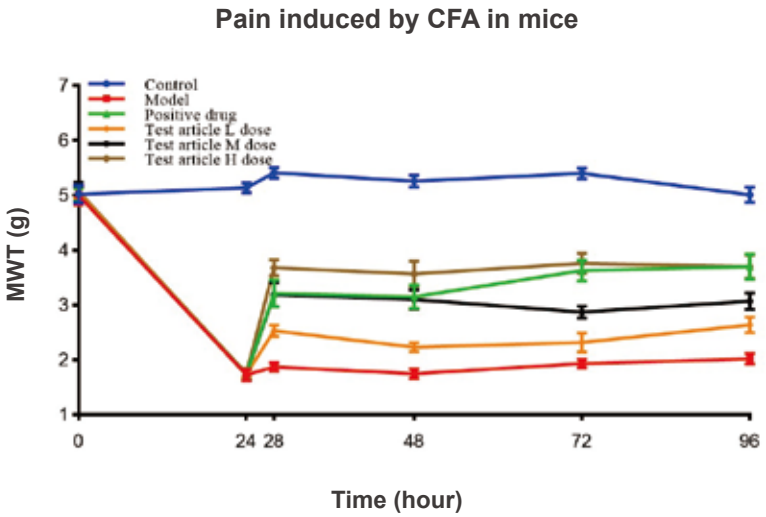




CFA Induced Inflammatory Pain Model

Complete Freund's Adjuvant (CFA) is a bacterial antigens-emulsifier routinely used to study inflammatory pain, as it mimics inflammatory pathologies, such as rheumatoid arthritis, in addition to presenting high reproducibility in rats and mice. CFA contains lipoproteins, glycolipids, and peptidoglycans that are recognized by antigen-presenting cells through pattern recognition receptors (PRRs). CFA induces peripheral and central sensitization of nociceptive neurons that can lead to painful conditions such as hyperalgesia and allodynia to thermal and mechanical stimuli. Thus, the CFA model can be established to investigate the potential mechanism for chronic inflammation pain. The model of CFA-induced peripheral inflammatory pain generates a state of persistent inflammation.

Medicilon Case: Complete Freund's Adjuvant (CFA) induced inflammatory pain model in mice

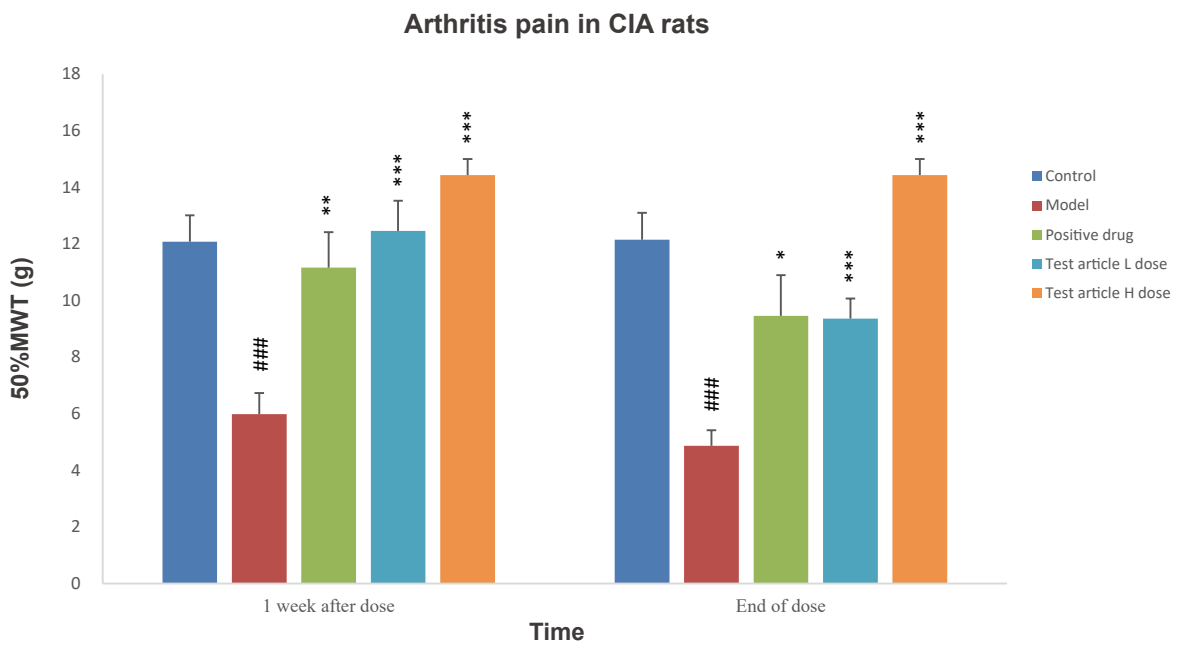


Collagen Induced Arthritis (CIA) Model

Rheumatoid arthritis (RA) is one of the most common chronic inflammatory diseases. RA is characterized by chronic joint inflammation, synovial hypertrophy, and progressive destruction of cartilage and bone that lead to debilitating joint pain and severe disability. The clinical symptoms, such as pain and difficulty to move inflamed joints, negatively affect the patients' well-being and ability to work, and induce psychological distress.Animal models of RA are essential for studying the pathogenesis of RA *in vivo* and determining the efficacy of anti-RA drugs.During the past decades, numerous rodent models of arthritis have been evaluated as potential models and the modeling methods are relatively well-developed.

Among these models, the CIA rat model has been extensively studied because it shares several pathological and immunological features with the human pathology, and allows testing innovative treatments in preclinical studies. In the CIA model, arthritis development and severity are assessed using a clinical scoring system based on peripheral joint swelling and redness.

Medicilon Case: CIA arthritis pain in rats

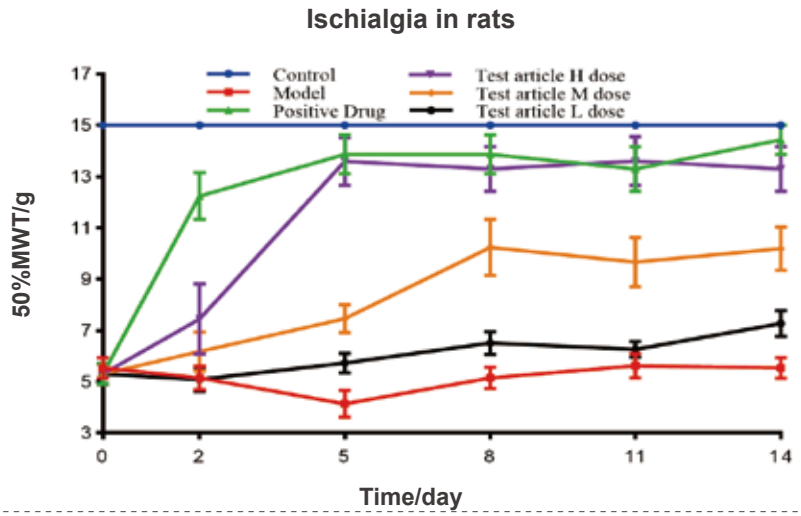


Sciatic Nerve Injury (SNI) Model

Peripheral neuropathic pain is a severe chronic pain condition which is produced by multiple etiological factors that initiate a number of diverse mechanisms operating at different sites and at different times and expressed both within, and across different disease states.

The sciatic nerve injury (SNI) model induces symptoms of neuropathic pain. Sciatic nerve ligation model is a partial ligation of the high sciatic nerve in rodents to simulate the symptoms of peripheral neuralgia and evaluate analgesic drugs acting on peripheral neuralgia, the SNI model may provide an additional resource for unraveling the mechanisms responsible for the production of neuropathic pain.

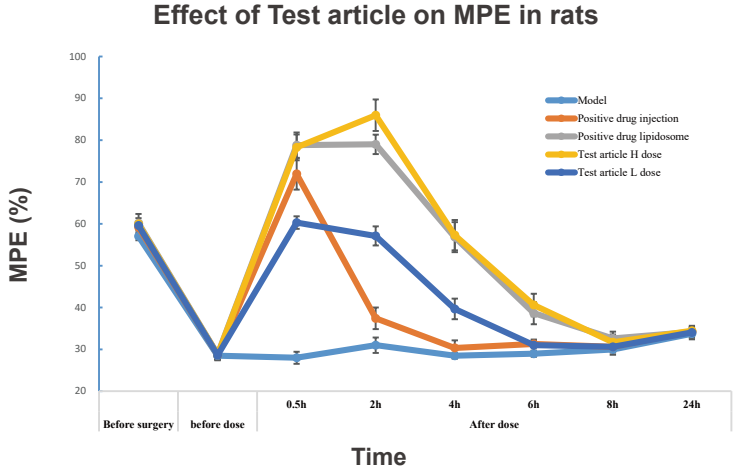
Medicilon Case: Sciatic nerve injury (SNI) model



Incisional Pain Model

Postoperative pain (POP) is a significant, worldwide problem. POP is highly debilitating and hinders recovery. POP is a transient but extremely strong activation of the nociceptive network. Opioids are the main pain medication used for acute postoperative pain. Given the addictive nature of opioid, non-opioid pain therapeutics are needed. Therefore, identifying the mechanisms that underlie acute postoperative pain is necessary for the development of optimal therapies for postoperative pain that may ultimately decrease the severity and/or incidence of chronic postoperative pain. The gold standard for POP is the Brennan model (incisional pain) at the preclinical stage. Nociceptive behaviors can be measured after incisional procedure, using notably the Von Frey test. Both rat and mouse models of acute incisional pain have been developed as preclinical models to research the molecular, cellular and physiological mechanisms that underlie postoperative pain. This model of postoperative pain is frequently-used, highly reproducible.

Medicilon Case: Incision-induced pain model

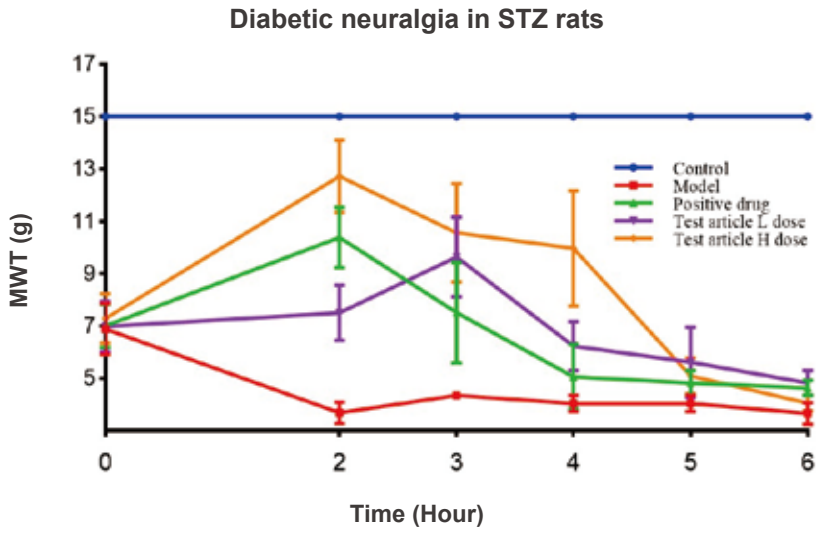


Diabetic Neuralgia Model

Diabetes is a metabolic disorder characterized disease by high blood glucose. Diabetic peripheral neuropathy (DPN) is the most common complication of diabetes and is associated with significant morbidity and mortality. DPN is characterized by a progressive distal-to-proximal degeneration of peripheral nerves, which results in sensory symptoms, including pain, weakness, and/or loss of sensation.

In vivo models are critical for understanding DPN pathophysiology and elucidating treatment strategies. In general, chemical induction, nutrition induction, and genetic modification are the three main strategies for establishing diabetic neuropathy models in rodents. Several established diabetic mouse and rat models exhibit neurological impairments associated with DPN, and new diabetic mouse strains that are more physiologically relevant to the human disease continue to be explored.

Medicilon Case: Diabetic neuralgia model

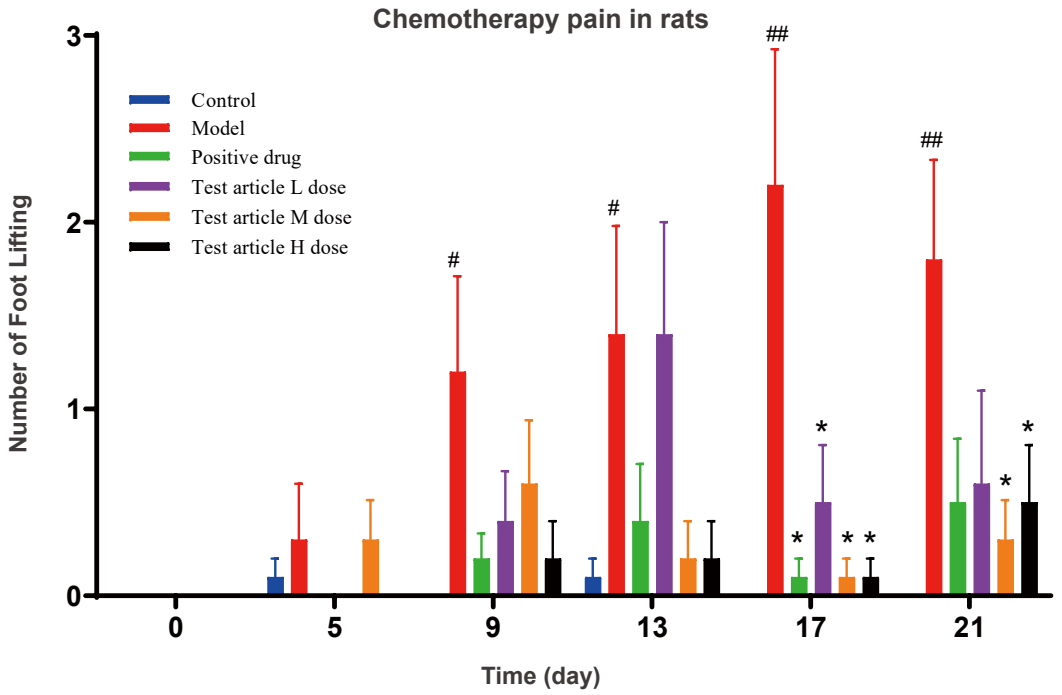


Chemotherapy Induced Pain Model

Chemotherapy-induced painful neuropathy (CIPN) is a major dose-limiting side-effect of several widely used chemotherapeutics. A side-effect of many chronic chemotherapy treatments in humans is development of peripheral neuropathy and its associated neuropathic pain. CIPN results in a range of predominantly sensory, bilateral symptoms in both hands and feet (also described as a stocking and glove distribution) including numbness, tingling, ongoing/spontaneous pain, hypersensitivity to mechanical and/or cold stimuli. CIPN is commonly observed following treatment with chemotherapeutics that have different mechanisms of anti-cancer actions, such as platinum agents, vinca alkaloids, taxanes, thalidomide, proteasome inhibitors and epothilones.

This condition can be replicated in a translatable fashion in rodents. Rodent models of CIPN have been developed by using a range of chemotherapeutic agents (including paclitaxel, vincristine, and others) to reproduce pain-like behaviors akin to patient-reported symptoms. The induced neuropathy presents as lower thresholds for mechanical sensation, cold sensitivity as well as decreased coordination and balance.

Medicilon Case: Chemotherapy pain model in rats



References:

[1] Adriana Domínguez-Oliva, et al. The neurobiology of pain and facial movements in rodents: Clinical applications and current research. Front Vet Sci. 2022 Sep 29:9:1016720. doi: 10.3389/fvets.2022.1016720.

[2] Amanda Gunn, et al. The influence of non-nociceptive factors on hot-plate latency in rats. J Pain. 2011 Feb;12(2):222-7. doi: 10.1016/j.jpain.2010.06.011.

[3] Colleen R McNamara, et al. TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci U S A. 2007 Aug 14;104(33):13525-30. doi: 10.1073/pnas.0705924104.

[4] Carlos Minoru Omura, et al. Decrease of IL-1β and TNF in the Spinal Cord Mediates Analgesia Produced by Ankle Joint Mobilization in Complete Freund Adjuvant-Induced Inflammation Mice Model. Front Physiol. 2022 Jan 14:12:816624. doi: 10.3389/fphys.2021.816624.

[5] Anne-Laure Mausset-Bonnefont, et al. Arthritis sensory and motor scale: predicting functional deficits from the clinical score in collagen-induced arthritis. Arthritis Res Ther. 2019 Dec 4;21(1):264. doi: 10.1186/s13075-019-2047-z.

[6] Ashley M Cowie, et al. A Mouse Model of Postoperative Pain. Bio Protoc. 2019 Jan 20;9(2): e3140. doi: 10.21769/BioProtoc.3140.

[7] Phillipe D O'Brien, et al. Mouse models of diabetic neuropathy. ILAR J. 2014;54(3):259-72. doi: 10.1093/ilar/ilt052.

[8] Holly L Hopkins, et al. Chemotherapy-induced painful neuropathy: pain-like behaviours in rodent models and their response to commonly used analgesics. Curr Opin Support Palliat Care. 2016 Jun;10(2):119-128. doi: 10.1097/SPC.0000000000000204.

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