REVIEW ARTICLE

Human experimental pain models: A review of standardized methods in drug development

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Human experimental pain models are essential in understanding the pain mechanisms and appear to be ideally suited to test analgesic compounds. The challenge that confronts both the clinician and the scientist is to match specific treatments to different pain-generating mechanisms and hence reach a pain treatment tailored to each individual patient. Experimental pain models offer the possibility to explore the pain system under controlled settings. Standardized stimuli of different modalities (i.e., mechanical, thermal, electrical, or chemical) can be applied to the skin, muscles, and viscera for a differentiated and comprehensive assessment of various pain pathways and mechanisms. Using a multimodel–multistructure testing, the nociception arising from different body structures can be explored and modulation of specific biomarkers by new and existing analgesic drugs can be profiled. The value of human experimental pain models is to link animal and clinical pain studies, providing new possibilities for designing successful clinical trials. Spontaneous pain, the main compliant of the neuropathic patients, but currently there is no human model available that would mimic chronic pain. Therefore, current human pain models cannot replace patient studies for studying efficacy of analgesic compounds, although being helpful for proof-of-concept studies and dose finding.

Key words: Analgesics, multimodel–multitissue, pain models, proof-of-concept, spontaneous pain

INTRODUCTION

Pain is the most prevalent health care problem, and characterization of pain is of major importance in the diagnosis and choice of treatment.[1] In clinical practice, the different symptoms of the underlying disease or complaints relating to psychological, cognitive and social aspects of the illness, as well as systemic reactions such as fever and general malaise confound the characterization of pain.[2] This may bias the clinical evaluation in assessing the efficacy of analgesics; also, repeated exposures to drug make patients familiar with its side effects and increase the chance of "active placebo" effects.[3] Because of these limitations, human experimental pain models are often helpful in preclinical studies of new analgesics. Also, in human models, the investigator can control the experimentally induced pain, including the nature, localization, intensity, frequency and duration of the stimulus and provide quantitative measures of the psychological, behavioral or neurophysiological responses.[4]

Most of the animal models of pain rely on detecting a change in the threshold or response to an applied stimulus and the absence of verbal communication in animals is undoubtedly an obstacle to the evaluation of pain. Also, the neurobiology of nociceptive systems differ between species and this limits the extrapolation of findings from animal studies to man.[5] However, the animal models can be used as tools to help to find-out the relative contribution of different pain mechanisms in changing an animal's behavior in a given situation.[6]

Human experimental pain models can act as a translational bridge between animal and clinical research and many of the mechanisms tested in animals can also be translated [Figure 1] and evaluated in healthy volunteers and used to predict the efficacy of a given drug in specific patient populations[4] [Figure 2]. Finally, reproducibility of the method is an important factor; if reproducibility is good, the model can be useful in drug screening[7] and randomized controlled trials are the ideal to explore the effectiveness of the clinical intervention.[8]

Assessment of the output from these pain models can be based on psychophysical or neurophysiological methods.[9] Psychophysical methods are the simplest way to assess the

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pain response[10] and are based on subjective experience of pain, measured on standard scales or as pain thresholds and neurophysiological methods include measurement of evoked brain potentials or nociceptive withdrawal reflexes.

DEFINITIONS

Allodynia

Pain due to a stimulus which does not normally provoke pain [Figure 3].

Hyperalgesia

An increased response to a noxious stimulus, caused by sensitization of peripheral nociceptors (primary hyperalgesia: The area of tissue injury) and/or by sensitization of central neurons (secondary hyperalgesia: outside the area of the original tissue injury) [Figure 3].

Temporal Summation/Windup Like Pain

It is a phenomenon that occurs when a repetition of a stimulus increases pain perception.

Spatial summation

It is a phenomenon that occurs when a non-painful stimulus is perceived as painful when applied to a wider area.

Referred pain

Pain that is felt in a part of the body at a distance from the area of stimulation.

PAIN-INDUCTION METHODS

Pain models can be classified according to their presumed mechanism (inflammatory vs neuropathic), the involved tissue (skin vs muscle vs viscera) and their time course (phasic – shorter, lasting for milliseconds to seconds vs tonic – longer, lasting for minutes). However, mechanistically the most important categories are peripheral or central sensitization. Conceptually, the two forms of sensitization are strictly separated; but, most of the pain models are characterized by a combination of peripheral and central sensitization. For example, cutaneous freeze injury lead to both allodynia and hyperalgesia.[11] The present review gives a brief overview of the pain models according to the tissue type, with an intention for the development of the sensitive pain model methods based on the knowledge of earlier methods described in the present review.

MECHANICAL STIMULATION

Mechanical stimulation of the skin

Mechanical stimulation of the skin can be grouped into Touch, Pin Prick, and Pinching. This method has been used to evaluate

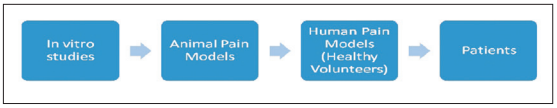

Figure 1: Human experimental pain models: Focus on translation

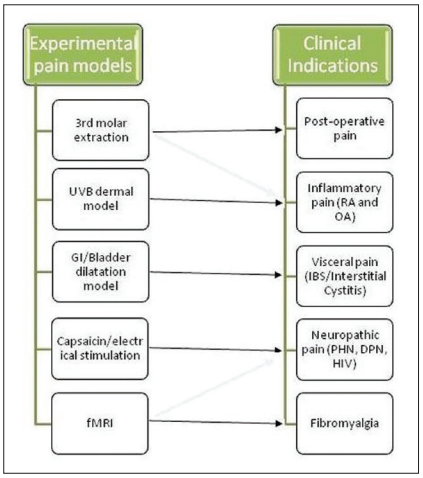

Figure 2: Examples for the scope of pain indications

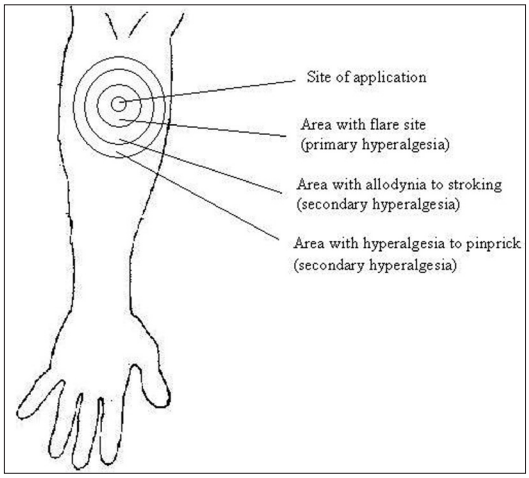

Figure 3: Illustrates hyperalgesia; manifested locally (primary hyperalgesia) and by central sensitization (secondary hyperalgesia), for example, after freeze injury or 30 minutes after application of capsaicin

the effect of clonidine, oxycodone, morphine, mepivacain, bupivacain, gabapentin, carbamazepine, and amitriptyline.

Touch

Sensitivity to touch can be assessed by applying a light pressure with a finger[12] or using a von Frey hair.[13] A-beta fiber mediate touch sensation.[14]

Major shortcomings

The von Frey hair activates both low threshold mechanoreceptors and nociceptors and is not specific.[6] Also, touch is used mainly to explore allodynia evoked by other pain stimuli.[15]

Pin-prick

Stimulation of the skin gently with a needle[16] or a safety pin[17] or thick von Frey filament and pain is reported as pricking or "first pain".[15] Pin-prick stimulation predominantly activates A-delta fibres.[14]

Major shortcomings

The rapidity of pain onset and termination is not easily controlled.[6,18]

Pressure

A skin flap can be squeezed between two pressure sensors[19] or a finger, toe[20] or an ear lobe[16] can be pinched between the algometer probe and a pinch handle.

Major shortcomings

The rapidity of pain onset and termination is not easily controlled.[6,18] Pain during pinching is a combination of mechanical stimulation and local ischemia.[21]

Mechanical stimulation of the muscle

This is typical exogenous experimental pain model and has been used for drug assessment of morphine, oxycodone, rofecoxib, tramadol, codeine, imipramine, and ketamine.

Pressure algometry is the most frequently applied technique for quantification of pain, where the probe is applied to a hard body structures, such as periosteum[16] or soft tissue such as muscles.[19] Both A-delta and C-fibers mediate pain induced by pressure stimulation.[22]

Major shortcomings

The technique is nonspecific since receptors in the skin, and probably deeper tissues will be activated.

Mechanical stimulation of the viscera

Mechanical stimulation of viscera has been used to evaluate the effect of serotonin $(5\text{-HT})_4$ receptor agonist tegaserod, as well as oxycodone, morphine, Octreotide (a somatostatin analog), nifedipine, NMDA receptor antagonist ketamine, tricyclic antidepressants like imipramine, amitriptyline.

Mechanical stimulation in viscera using balloon distension has been widely used to study pain perception thresholds, referred pain and cerebral activation patterns[23] as well as, to screen new analgesics in healthy subjects and those with gut disorders.[24,25] The introduction of the electronic barostat, has helped to ensure proper location of the balloon, regardless of the inflation paradigm that was used. The newer methods based on impedance planimetry, allows recording of the luminal cross-sectional area directly and estimation of the radius in the distended segment of the esophagus or intestine.[26]

Major shortcomings

Unlike the rectum and the stomach, the esophagus doesn't serve as a storage organ, but rather as a conduct. Consequently intraesophageal distensions do not mimic a normal, physiologic stimulus and thus perceptual responses to such a stimulus may have no scientific merit. In addition, difficulties in tolerating balloon distension, commonly results in poor recruitment rates as well as the potential for esophageal perforation, have made esophageal balloon distensions by a barostat a less attractive research tool.

THERMAL STIMULATION

Thermal stimulation of the skin

A cold stimulation/cold pressor pain. Application of ice,[27] a cold gel bag,[28] a wet alcohol sponge[12] menthol[29,30] ether, or a Peltier thermode to the skin evokes cold sensation. The method has been intensively used for drug screening such as imipramine, paroxetine, morphine, codeine, tramadol, and oxycodone.

Nociceptors of cutaneous veins appear to mediate cold pain in humans[31] via activation of A-delta and C-fibres.[32] However, a lack of standardization in the cold pressor model is revealed, with substantial variation in both equipment and methodology used.[33]

Major shortcomings

Vascular reactions strongly affect the response and cold pressor pain has shown contradictionary results in the testing of analgesics.[7] Also, pain onset and offset are gradual, and the experiment is not repeatable within a 1--2 hour session.[18]

Freeze lesion

Application of cold temperatures (-20°C) at a standardised pressure provides stable testing conditions for 1 day.[34]

Major shortcomings

A central component of the analgesia can be explored, even if the method is thought to evoke mainly peripheral mechanisms.[35]

Contact heat

The most commonly used heat/cold contact stimulators are based on the Peltier principle (one direction of the current causes cooling and the other way heating).[36] Warm sensation is mediated by C-fibers.[37,38] At threshold determinations, rapid skin heating activates first A-delta fibres, corresponds to the "first pain" followed by a C-fibre mediated second pain, being described as 'throbbing, burning or swelling'.[39] Slow heating gives a preferential activation of C-fibers (thought to be most important for peripheral opioid receptors) and the best evaluation of

second pain.[7] In order to prevent injuries to the skin, the high temperature limit is recommended to be 50 °C and the low limit 5 °C.[40]

Major shortcomings

Contact of the thermode with the skin activates concomitant low-threshold non-nociceptors. The rate of thermal transfer depends on the thermode-skin contact and thus on the pressure of application of the thermode. Therefore it is important that the thermode is applied to skin at a standardized fashion.

Radiant heat

Laser pulses evoke a distinct pricking pain in skin.[40,41] Intensities higher than those evoking pricking pain are avoided, as they may cause superficial burns.[42] Depending on the stimulus intensity, laser-induced thermal stimulation produces a double pain sensation: an initial prick sensation attributable to A-delta fiber activity[41] and a second diffuse burning sensation due to C-fiber activity.[43] Different laser emission sources have been developed in pain research e.g. argon, copper vapor[44,45] thulium-YAG and laser diodes, but CO_2-laser[46] is most commonly used.[40] The argon[44] and copper vapor lasers[45] operate, a different principle than CO_2-laser, where in CO_2-laser, radiation is absorbed within the epidermis, independent of skin pigmentation and application angle. Whereas, in the Argon-laser much of the radiant heat is reflected, causing variation depending on the skin pigmentation and the application angle.[47] In all laser studies, the spot of stimulation has to be shifted slightly between consecutive stimuli in order not to cause receptor sensitization/fatigue. Irradiation using ultraviolet B (UVB), produces stable areas of primary hyperalgesia over several hours.[48,49]

Major shortcomings

Variability in responses between the individuals was observed.[6,47]

Burn injury

The burn injury model illustrates hyperalgesia. Contact heat and radiant heat can induce burn injury by, e.g., application of a constant temperature of 47 °C for 5 min (leads to long lasting sensitization). The brief thermal sensitization model (BTS; 45 °C for 3 min) provides short lasting sensitization and can be induced 2–3 times at hourly intervals without skin injury. Among the endogenous inflammatory mediators, prostaglandin E2 may be responsible for early heat hyperalgesia.[50] Whereas in longer lasting mechanical hyperalgesia nerve growth factor may be involved.[51,52] Both A-delta and C-fibres mediate pain after a burn injury.[15]

Major shortcomings

The threshold for activation of mainly A delta-fibres may transform into thresholds for activation of C-fibres. As these respond to other stimulus modalities it may confuse the testing of an analgesic.[6]

Thermal stimulation of the muscle

Warm and cold pain has been evoked from muscle tissue when saline at different temperatures is injected intramuscularly.[53]

Major shortcomings

No drug studies have been performed.

Thermal stimulation of viscera

Phasic thermal stimuli of the human gastrointestinal tract are believed to activate unmyelinated afferents in the mucosa selectively. This is opposed to mechanical and electrical stimuli, which activate afferents in both superficial and deep layers.[54] Thermosensitive mucosal afferents have been demonstrated in the human esophagus, stomach and rectum.[26,54] Human gastrointestinal tract shows a uniform perception of thermal stimuli with different reflex responses from the stomach to the jejunum.[55] The temperature of recirculating water, when continuously measured inside a balloon positioned in the esophagus, showed a linear stimulus–response relationship, demonstrating the validity of the activation.[26]

Major shortcomings: Only a few pharmacological studies have been performed using thermal visceral stimulation (e.g., oxycodone and morphine).

ELECTRICAL STIMULATION

Electrical stimulation of the skin

Various electrical stimulator devices connected to electrodes applied either to the skin surface[28] or the intracutaneous tissue[56] evoke electrical stimulation. Stimulator devices can deliver different stimulation pattern, for example, different waveforms, frequencies and duration of stimuli. This activates with some selectivity different afferents and nervous structures, and hence evokes different kinds of pain.[6,7] Electrical stimulation excites nerve fiber populations and the relative proportion of activation of individual fiber types depends on the stimulus intensity. [7] C-fibers have a higher activation threshold than A-delta fibers. Drug studies on drug classes like opioids, tricyclic antidepressants and NSAIDs have been performed using electrical stimulus of different intensities.

Major shortcomings

Electrical stimulation bypasses the receptors and activates

the nerve fibers directly, and the method is not a specific activation of the nociceptors. The electrical threshold is related to the fiber diameter and one cannot usually excite small-diameter nerves without additionally exciting others.

Electrical stimulation of the muscle

Electrical stimulation of the muscle can be performed via small needle electrodes with un-insulated tips.[57] Repeated electrical stimulation can induce temporal summation and cause increase in referred pain areas, thus reflecting central changes.[58,59] The technique has been used to evaluate drugs such as remifentanil, morphine, alfentanil, oxycodone, and ketamine.

Major shortcomings

Electrical stimulation is not nociceptive specific as it bypasses the receptors. Furthermore, concurrent activated muscle twitches may confound the sensation evoked by intramuscular electrical stimulation.[60]

Electrical stimulation of the viscera

Electrical stimulation of the gut has been widely used to study basic pain mechanisms, pain characteristics, referred pain and evoked brain potentials. The use of electrical stimulation has demonstrated safety in all parts of the gastrointestinal system. Furthermore, the well-defined onset and offset of the stimulation eliminates the latency as observed with other methods, making this particularly suitable for neurophysiological assessments.[2,61] The technique has been used successfully in drug studies of valdecoxib, parecoxib, morphine, oxycodone, and ketamine.

Major shortcomings

Electrical gut stimulation, bypasses the receptors and activates the nerve fibers directly. The major drawback with earlier methods was the varying electrode contact with the mucosa, giving inconsistent results.

CHEMICAL STIMULATION

Chemical stimulation of the skin

Capsaicin

Intradermal injection or topical application of capsaicin directly evokes pain, and hyperalgesia. [62] Capsaicin induced pain has been suggested as a surrogate model of changes observed in neuropathic pain. Mostly C-fibres are thought to mediate pain.[63] Capsaicin application activates transient receptor potential vanilloid 1 (TRPV1) receptors. This model is widely used for drug evaluations, such as the effect of neurotoxins (e.g., Botox) on TRPV1 sensitive nociceptive endings, as well as ketamine, magnesium, lidocaine, alfentanil, diclofenac, orphenadrine, gabapentin, cannabis, lamotrigine, H_1 antagonists, hydromorphone, and the lidocaine patch.

Major shortcomings

Variable response was obtained with the model, e.g., pharmacological testing of lamotrigine and desipramine which are used in the treatment of neuropathic pain failed to show any effects in the model.[63,64] On the other hand gabapentin, which is also used to treat neuropathic pain, suppresses hyperalgesia following heat-capsaicin sensitization.[65]

Mustard oil

Topical application of mustard oil induces pain and hyperalgesia. C-fibres are thought to mediate the burning pain, while A-beta fibers are believed to mediate allodynia to light mechanical stimuli.[15]

Major shortcomings

The method has not been used much in the testing of analgesics. The use of these models is basically limited to target engagement studies.

Chemical stimulation of muscle

Intramuscular infusion of hypertonic saline[59,66] glutamate[67] and capsaicin[68] induces pain and referred pain areas. Hypertonic saline mimics musculoskeletal pain in both subjectively perceived quality as well as its effects on motor performance.[69] The dominant sensation following hypertonic saline injections in the muscle is a deep and diffuse pain, via activation of C-fibres. Earlier manual bolus infusions of hypertonic saline were used. Standardization of a small bolus volume is easy to accomplish by a computer-controlled infusion pump. This provides more reproducible method[60] and have been used to evaluate the effects of the NMDA receptor blocker, ketamine, as well as morphine and alfentanil. Intramuscular injections of algesic substances such as bradykinin[70], serotonin[70], substance P[70], potassium chloride, L-ascorbic acid, and acid phosphate buffer are other chemical stimulation methods to evoke muscular pain.

Major shortcomings

Hypertonic saline injections may excite both non-nociceptive and nociceptive nerve fibers. The chemical stimulation methods all have a problematic reproducibility with large interindividual differences.[71]

Chemical stimulation of the viscera

Chemical stimulation is believed to be the ideal experimental visceral pain stimulus, closely resembles clinical inflammation. Acid stimulation of the esophagus is the most common method to sensitize the gut.[72] Application of glycerol to the large intestine evoked pain in patients

with the irritable bowel syndrome.[73] In the colon mucosa, injections of 2%--6% hypertonic saline resulted in deep as well as referred nonpainful and painful perceptions.[2] Most chemical stimuli are believed to activate predominantly unmyelinated C-fibers.[74] Application of capsaicin in the ileum resulted in a dose-dependent pain response and referred pain.[2] Chemical stimulation of gut using algogenic substances like alcohol, bradykinin has also been performed in humans.[2]

Major shortcomings

The major disadvantage of chemical stimulation is a relatively long latency time to the onset of effects and often responses are not reproducible when repeated.

ENDOGENOUS METHODS OF MUSCLE STIMULATION

Ischemic stimulation

The tourniquet model is a classical experimental pain model that induces ischemic muscle pain. Earlier methods of producing experimental pain by occluding the blood flow of exercising muscles failed to demonstrate satisfactory response. The modified method developed by smith *et al*.[75] more closely resembles pain of pathologic origin. It has been used in human analgesic assays such as morphine, tramadol, caffeine, rofecoxib, aspirin, ibuprofen. This model is applicable in experimental studies requiring a general tonic pain stimulus.[60]

Major shortcomings

It is a very efficient model to induce pain in the muscles but is non-specific, since skin, periosteum, and other tissues will contribute to the overall pain perception. When activating nociceptors, concomitantly low-threshold non-nociceptive nerves can be activated by the contact of the tourniquet with the skin. This activation can exert an inhibitory influence on pain mechanisms.

Pain evoked by exercise

Delayed onset muscle soreness (DOMS) is a sensation of muscular pain during active contractions or passive stretch of a muscle after unaccustomed or eccentric exercise, which peaks 24–48 hours after exercise.[76] DOMS is thought to be caused by structural damage to muscle that leads to the release in the muscle of algogenic substances such as prostaglandins.[77-79] These algogenic substances sensitize A-delta and C nociceptive fibres.[79] Large mechanoreceptor afferents from muscle, muscle spindle and tendons are activated in DOMS, and may also contribute to the pain syndromes.[80-84] This model was used for drug evaluations, such as morphine, tramadol, codeine, ketoprofen, diclofenac, ibuprofen, rofecoxib and naproxen.

Major shortcomings

Neural mechanisms that leads to DOMS were incompletely understood. Variable results were obtained.[85-87] Animal studies show that a stress-induced analgesia can occur with eccentric exercise.[88] These also bias the results in analgesic testing.

SCOPE OF TRANSLATIONAL PAIN RESEARCH

Traditionally, translational research is regarded as a process of bridging bench findings to clinical application and the process requires coordinated bidirectional approaches between bedside and bench because of the subjective nature of the pain. This is an advantage in proof-of-concept studies, where the efficacy of a given compound on specific mechanisms can be assessed and the dose-response relationship can be determined.

The link between sensitization in inflammatory models such as UVB burn and clinical inflammatory conditions is considerably good. Also, a new model of peripheral and central sensitization without inflammation: intracutaneous injection of nerve growth factor[89] which appears to generate a combination of symptoms similarly found in patients. However, even these models only reflect part of the disease. Still we lack information about the role of trophic factors for the long-term modulation of nociceptor structure and sensitivity. Therefore, current theories or concepts of pain mechanisms need to be critically reviewed and analyzed to provide a new roadmap of contemporary pain research.

Spontaneous pain is a salient feature of clinical pain, which is the main complaint of neuropathic patients, reported to have more severe depression and physical disability[90] is not mimicked in most of the human pain models.[10] This essential gap is based on ethical limitations of human models according to which no healthy volunteers can be turned into a chronic pain patient. Irrespective of this limitation, the mechanism leading to spontaneous pain is unknown and even the site of origin is debated.

Finally, the pain arising from the skin, muscles and viscera differ from one another, and hence compounds may show very different effects on pain from these structures. Therefore, the concept of multimodel, multitissue pain assessment has been developed, where advanced drug screening in healthy volunteers and patients is possible. Therefore, a meaningful translation process through clinical studies should begin with careful choices of appropriate clinical pain conditions that are consistent with the conditions examined in preclinical models.

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