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REVIEW

Predictive validity of behavioural animal models for chronic pain

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Rodent models of chronic pain may elucidate pathophysiological mechanisms and identify potential drug targets, but whether they predict clinical efficacy of novel compounds is controversial. Several potential analgesics have failed in clinical trials, in spite of strong animal modelling support for efficacy, but there are also examples of successful modelling. Significant differences in how methods are implemented and results are reported means that a literature-based comparison between preclinical data and clinical trials will not reveal whether a particular model is generally predictive. Limited reports on negative outcomes prevents reliable estimate of specificity of any model. Animal models tend to be validated with standard analgesics and may be biased towards tractable pain mechanisms. But preclinical publications rarely contain drug exposure data, and drugs are usually given in high doses and as a single administration, which may lead to drug distribution and exposure deviating significantly from clinical conditions. The greatest challenge for predictive modelling is, however, the heterogeneity of the target patient populations, in terms of both symptoms and pharmacology, probably reflecting differences in pathophysiology. In well-controlled clinical trials, a majority of patients shows less than 50% reduction in pain. A model that responds well to current analgesics should therefore predict efficacy only in a subset of patients within a diagnostic group. It follows that successful translation requires several models for each indication, reflecting critical pathophysiological processes, combined with data linking exposure levels with effect on target.

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Abbreviations
CCI, chronic constriction injury; FCA, Freund's complete adjuvant; MIA, monosodium iodoacetate; NSAID, non-steroidal anti-inflammatory drug; PSL, partial sciatic nerve ligation; SNI, spared nerve injury; SNL, spinal nerve ligation; TST, tibial and sural nerve transection

Introduction

Chronic pain affects 20–30% of the adult population in western countries, and the medical need for more efficacious, better-tolerated and safer analgesics is undisputed (Smith *et al.*, 2001; 2007; Portenoy *et al.*, 2004; Rice and Hill, 2006; Finnerup *et al.*, 2010; Johannes *et al.*, 2010). Accordingly, discovery and development of novel analgesics is subject to extensive research in both academia and industry. Rodent behavioural pain models are used at all stages of this work, from identification of novel targets and mechanisms to estimation of efficacy and therapeutic window.

The modelling of chronic pain does, however, pose some specific challenges. Pain is a subjective, multidimensional experience with sensory, emotional and cognitive components that are difficult, if not impossible, to incorporate into a single animal model. Interpretation of animal behaviour in terms of specific sensory modalities and affective connotations is far from straightforward. From the clinical perspective, chronic pain is heterogeneous, even within diagnostic categories, and these categories are defined according to anatomical and aetiological criteria of limited value when it comes to understanding the factors causing pain in individual patients (Jensen and Baron, 2003). The heterogeneity

of clinical pain is reflected in the response to pharmacotherapy. In neuropathic pain, the majority of patients in clinical trials receive less that 50% pain relief compared with placebo regardless of which drug they receive (Finnerup *et al.*, 2010). The situation for acute postoperative pain (Derry *et al.*, 2009), chronic joint pain (Towheed *et al.*, 2006; Cepeda *et al.*, 2007) and chronic low back pain (Martell *et al.*, 2007; Machado *et al.*, 2009) is similar. It is clear that any single current analgesic treatment is effective in only a subset of patients within a diagnostic category. The implication for animal modelling aiming at prediction of analgesic efficacy and potency is that a single model is likely to represent only a certain set of pathophysiological mechanisms or at best a patient segment, which may not correspond to a traditional diagnostic category. This concept is fundamentally different from a historical and still not uncommon view that a predictive model should be generally applicable across pain conditions, or at least within a category such as neuropathic pain or pain related to osteoarthritis (Taber, 1974).

The overall challenge is therefore to understand which mechanisms of chronic pain a particular model can address and how it correlates to signs and symptoms in defined segments of patients. The construct validity of the model is, in other words, critical. A model needs to demonstrate both sensitivity (i.e. the ability to predict analgesic efficacy) and specificity (i.e. ability to detect negative outcomes) to be useful in predicting efficacy and potency. There are, however, great gaps in our understanding of pain pathophysiology, and it is not clear how patient segmentation for optimal treatment can best be achieved (Serra, 2010), let alone modelled. We will here review some commonly used models from this perspective. More comprehensive discussion of animal models can be found in a number of recent reviews (Decosterd and Berta, 2008; Di Paola and Cuzzocrea, 2008; Jeong and Holden, 2008; Pacharinsak and Beitz, 2008; Authier *et al.*, 2009; Bradesi and Mayer, 2009; Sandkühler, 2009; Sorkin and Yaksh, 2009; Colleoni and Sacerdote, 2010).

Mechanistic approaches, acute pain models and assays of physiological nociceptive pain

Nociceptive pain tests based on withdrawal responses elicited by stimuli at or near threshold intensity, for example, thermal assays like the tail flick and hot plate tests, are frequently referred to as models of acute pain. This is confusing since acute pain in clinical terminology typically refers to pain caused by accidental injury or surgery and involves a set of inflammatory and sensitizing mechanisms not present in the common implementations of these tests. These assays can be used to study physiological nociception, and their utilities and limitations have been extensively discussed elsewhere (Le Bars *et al.*, 2001). In terms of pharmacology, the acute assays are sensitive to what we now know are agonists at the μ opioid receptor but not to other types of opiates (Taber, 1974). The hot plate assay is, for instance, capable of predicting a potency ranking of spinal opioid analgesics corresponding to clinical dosing in postoperative pain (Yaksh, 1997). Usually, response latency is measured but use of ramped thermal stimuli may allow estimation of an approximate response threshold (Tjølsen *et al.*, 1991). These assays are not sensitive to non-opiate analgesics unless very high, perhaps toxic doses are administered (Taber, 1974; Liles and Flecknell, 1992).

Of greater face validity for acute pain in general and post-operative pain in particular are models of surgical incision with and without additional manipulations such as retraction of the tissue. Depending on the part of the body involved and surgical procedures, symptoms may last from several days in plantar incision models to several weeks in more complex models (for review, see Bove *et al.*, 2009 and Brennan *et al.*, 2005). Post-surgical hypersensitivity is generally quantified by means of von Frey filaments, but in some models, increases in heat and/or cold sensitivity can be demonstrated and quantified. The plantar incision procedure has been used extensively in pharmacological studies and has demonstrated efficacy of established analgesics and experimental compounds, in some cases dependent on stimulus modality (Brennan *et al.*, 2005).

A number of agents will, when injected intraperitoneally in rodents, produce a behavioural response consisting of stretching and writhing, utilized in the writhing test. Historically, various implementation of this test has shown good sensitivity to analgesics of different classes, but the specificity is poor (Taber, 1974). Used with agents that activate specific receptors or mechanisms, they may be useful for mechanistic studies. Irritants may also be administered to skin and other organs. The prototypical approach using capsaicin to activate TRPV1-expressing nociceptors has been applied to several species (Butelman *et al.*, 2004; Oliveira *et al.*, 2005; Joshi *et al.*, 2006; Sawynok *et al.*, 2006; Plevkova *et al.*, 2010), including man (Chizh *et al.*, 2009).

Subcutaneous injection of formalin induces behaviour suggestive of pain or irritation in several species and causes pain in man (for review, see Tjølsen *et al.*, 1992; Capone and Aloisi, 2004; Raboisson and Dallel, 2004). The formalin test is used routinely only in rodents where an injection of dilute formalin into the dorsum or plantar tissue of a paw causes a behavioural response consisting of licking, flinching and shaking of the injected paw. To study mechanisms related to the trigeminal system, orofacial versions have been developed (Clavelou *et al.*, 1989; Luccarini *et al.*, 2006). The concentration of formalin determines the time course of behaviour (one or two phases), the tissue response and the response to pharmacological treatment (Rosland *et al.*, 1990; Tjølsen *et al.*, 1992; Damas and Liégeois, 1999, Munro, 2009). Both phases of the behavioural response are associated with a primary afferent drive that would be expected to initiate and maintain activity-dependent sensitization at a spinal level. Central modulation occurs, at least in the second phase of the test (Dickenson and Sullivan, 1987; McCall *et al.*, 1996; Abbadie *et al.*, 1997). This phase is generally more sensitive to pharmacological intervention. Whether this is due to development of a different pathology or to differences in stimulation intensity between the phases is unclear. Testing animals beyond the usual time frame has revealed hypersensitivity to mechanical and thermal stimuli peaking 1–3 days after formalin injection and lasting up to 4 weeks (Fu *et al.*, 2001). It is therefore likely that full-blown inflammation follows a different time course than the spontaneous behaviour. Whether inflammation plays a significant role in driving the behavioural response during the second phase is uncertain, and the test should not be referred to as inflammatory unless

supported by other evidence, for example, biomarkers or relevant pharmacology, from experiments using an identical protocol. In general, the formalin test is sensitive to non-steroidal anti-inflammatory drugs and mild analgesics only in high doses. The test is probably best seen as a mechanistic model of pain driven by paroxysmal peripheral discharge, whether nociceptive or neuropathic in origin. It should be appreciated that there are many different implementations of the model, which makes it difficult to compare results across studies (Capone and Aloisi, 2004).

Models of chronic arthritis

Joint pain, particularly related to osteoarthritis, is a common indication for early clinical trials of new analgesics. Models of polyarthritis are primarily used for studies of disease and disease modification and are less suited for analgesic research due to the effects on the general health condition of the animals. The mainstay for modelling of symptomatic treatment of joint pain are models based on local injection of inflammatory agents, either into a knee or ankle joint or subcutaneously into the foot pad of a rodent. Intra-articular injection of Freund's complete adjuvant (FCA) was introduced as an arthritis model of longer duration than obtainable by urate crystal injections, but without the generalized disease produced by systemic injection of FCA (Butler *et al.*, 1992). In the original study, there were behavioural and clinical signs of arthritis between the second and sixth week after injection. A similar implementation displaying long-lasting hypersensitivity was published a few years ago (Wilson *et al.*, 2006). Relatively large amounts of FCA were injected in these studies, and although systemic effects were not observed, this remains a risk. More commonly used lower doses of FCA cause an inflammatory response lasting up to 2 weeks, which in most cases is sufficient for repeated administration of drugs. If a shorter duration is acceptable, carrageenan may be used as an induction agent, producing an arthritis that peaks within 4–8 h and resolves within 2–3 days (Schött *et al.*, 1994). FCA- and carrageenan-induced monoarthritis models are also feasible in mice, with a similar time course as in rats (Heilborn *et al.*, 2007).

A model claimed to be more disease-like as regards osteoarthritis is based on intra-articular injection of monosodium iodoacetate (MIA) (Kalbhen, 1987). This procedure leads to hypersensitivity for more than 4 weeks and, interestingly, is sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) only during the first few days (Fernihough *et al.*, 2004). There are also surgical models of osteoarthritis (Ameye and Young, 2006), not yet commonly used in the study of symptomatic treatments.

In these inflammatory models, hypersensitivity and drug effects are usually quantified by observational rating of paw pressure against the floor during standing or walking but even by evoked responses to thermal and mechanical stimuli such as paw withdrawal, vocalization and struggling (Coderre and Wall, 1987; Yu *et al.*, 2002). Several systems have been developed or modified to provide automatic or semiautomatic quantification of weight bearing (Schött *et al.*, 1994; Bove *et al.*, 2003) or changes in gait (Ängeby-Möller *et al.*, 2008). These methods may provide more graded and differentiated readouts than manual methods and facilitate objective scoring. Carrageenan and FCA can also be applied subcutaneously, usually into the plantar surface of the rat hind paw, which causes inflammation and hypersensitivity that can be quantified in a similar manner as in the monoarthritis models and follow a similar time course.

Models of peripheral mononeuropathy

The majority of animal studies on neuropathic pain rely on traumatic injury to a single nerve, usually the sciatic. A rat model of varicella zoster virus–associated neuropathic pain exists but is not widely used, and it is not clear how closely it models post herpetic neuralgia (Fleetwood-Walker *et al.*, 1999; Hasnie *et al.*, 2007). Rodent models of diabetic and chemotherapy-induced neuropathy are well established, but in some implementations, general health affection may interfere with analgesic modeling, and the models reflect clinical features to a variable degree (Wuarin-Bierman *et al.*, 1987; Fox *et al.*, 1999; Morrow, 2004; Authier *et al.*, 2009; Obrosova, 2009). We will here focus on the predominant partial sciatic nerve lesion models as originally developed in rats. Mouse versions of these models also exist (Gustafsson *et al.*, 2003; Bourquin *et al.*, 2006; Kiso *et al.*, 2008; Zhang *et al.*, 2008).

The chronic constriction injury model (CCI) is induced by loosely constrictive ligatures around the sciatic nerve trunk at mid-thigh level (Bennett and Xie, 1988). Animals show long-lasting changes in gait, posture, guarding and spontaneous lifting of the affected paw as well as reduced rate of body weight gain, which may indicate presence of spontaneous pain. Other features of the model are increased sensitivity to heat, cold and mustard oil, while deep pressure threshold was not altered in the original study. The partial sciatic nerve ligation model (PSL) was specifically developed to mimic causalgia (Seltzer *et al.*, 1990; Shir and Seltzer, 1990). Approximately 50% of the sciatic nerve trunk is tied off by tight ligation, which leads to increased sensitivity to heat and touch. In the original studies, neonatal capsaicin treatment prevented the development of thermal but not mechanical hypersensitivity, indicating differential involvement of myelinated and unmyelinated fibers. Involvement of the sympathetic system further indicated causalgia-like pathophysiology (Shir and Seltzer, 1991).

Several modifications of the partial sciatic lesion models have been published, primarily with the aim to simplify and standardize the procedures. Constriction by a polyethylene cuff instead of sutures leads to a shorter-lasting model (Mosconi and Kruger, 1996). Photochemically induced microinfarction has been reported to produce a higher frequency of pronounced tactile hypersensitivity compared with the CCI and PSL methods (Gazelius *et al.*, 1996; Cui *et al.*, 2000). In all three models, inflammatory cell count and pro-inflammatory cytokine levels were increased at the lesion site at 14 days after surgery, and the inflammatory response correlated with tactile hypersensitivity in the CCI and PSL animals (Cui *et al.*, 2000). Inflammation may play a role in the genesis of neuropathy in these models but may also be a confounding factor producing symptoms and pharmacological responses not exclusively related to neuropathy.

Another approach designed to facilitate a more standardized procedure for partial nerve lesion is the spinal nerve ligation (SNL) model, in which spinal nerves L5 and L6 are tightly ligated distal to the dorsal root ganglia (Kim and Chung, 1992). The authors found comparable increases in

sensitivity to noxious heat and mechanical stimuli as in the PSL model and noticed behaviours interpreted as signs of spontaneous pain (licking of the affected paw and overgrowth of nails). In a study comparing the CCI, PSL and SNL models, mechanical hypersensitivity was more pronounced in the SNL and least in the CCI model, while behavioural signs indicating ongoing pain were more prominent in the latter (Kim *et al*., 1997). The behavioural signs of neuropathic pain tended to decrease after sympathectomy in all models but most clearly in the SNL model.

Transection of different combinations of the three distal branches of the sciatic nerve (tibial, sural and common peroneal) induces long-lasting increases in sensitivity to mechanical and thermal stimuli as well as indications of spontaneous pain. Lee *et al*. (2000) reported that sectioning of the tibial and sural nerves while leaving the common peroneal nerve intact (TST) was more effective, rendering a model independent of sympathetic input. Ligation and transection of the tibial and common peroneal branches but sparing the sural branch, known as the spared nerve injury (SNI) model, allows investigation of sensory changes in the innervation territory of both injured and neighbouring intact sensory neurons (Decosterd and Woolf, 2000).

The various partial sciatic nerve lesion models differ somewhat in duration and magnitude of sensory changes, signs of spontaneous pain, but also in terms of technical difficulty and reproducibility (Dowdall *et al*., 2005). Although the main features of the models have been reproduced across a great number of laboratories, surgical skill and variations in procedure will impact the outcome of neuropathy models and contribute to variability (Zeltser *et al*., 2000). Even genetic differences are significant (Mogil *et al*., 1999; Shir *et al*., 2001; Xu *et al*., 2001). Taken together, these factors may be a greater source of variation than model-specific differences. A recent study investigating the effects of clinically used analgesics in the CCI and SNL models found that the efficacy depended more on readout than on model (Pradhan *et al*., 2010). Hypersensitivity to heat and pressure were highly responsive to oxycodone, gabapentin and amitriptyline, but the increased response to cold was only partially reversed. Even the increased sensitivity to mechanical stimulation with von Frey filaments was only partially reversible, and amitriptyline was ineffective on this parameter, in line with some, but not all studies cited by Kontinen and Meert (2003). In contrast, Whiteside *et al*. (2008) found no effect of amitriptyline on paw pressure sensitivity in the SNL model.

Models of persistent and chronic visceral pain

The writhing test is occasionally classified as a visceral pain model, but both visceral and somatic structures are activated by intraperitoneal injection of irritants, and the method has other limitations as discussed above. A large number of models specifically addressing visceral hypersensitivity has been developed, in most cases using distention of a hollow organ as stimulus and applying behavioural and electrophysiological readouts. This approach was introduced with the colorectal distension model in which aversive behaviour, cardiovascular and viceromotor responses were observed and extensively characterized in awake, unrestrained rats (Ness and Gebhart, 1988). Similar approaches have been applied to other viscera and complemented by pretreatment with inflammatory agents or neonatal stress inflicted by maternal separation or noxious stimulation to produce models of persistent and long-lasting visceral hypersensitivity (for reviews, see Joshi and Gebhart, 2000; Sengupta, 2009). Clinically used analgesics like opioids and clonidine have shown efficacy in several of these models. For a condition like irritable bowel syndrome, efficacy of novel drugs has been correlated with effects in human experimental models, but overall, the correlation has been weak between the hypersensitivity measures obtained in experimental models and clinical pain and global symptoms (Mayer *et al*., 2008).

Behavioural readouts

Typical readouts in the somatic pain models are responses to punctuate tactile stimuli applied with von Frey filaments (Chaplan *et al*., 1994; Le Bars *et al*., 2001), deep pressure (Randall and Selitto, 1957), heat (Hargreaves *et al*., 1988) or cold (acetone applied to the affected paw). Alternatives to the von Frey filaments are electronic devices that allow graded stimulation, may be easier to standardize and avoid some of the recognized drawbacks of the standard nylon filaments (Ängeby-Möller *et al*., 1998; Lever *et al*., 2003; Bove, 2006). Due to the progressive nature of the routinely used mechanical stimuli, initial activation of primary afferent fibers in the non-noxious range is a built-in feature, and withdrawal responses may or may not indicate hyperalgesia (Hogan *et al*., 2004). Cold sensitivity is sometimes measured by acetone spray (Dowdall *et al*., 2005) or ethyl chloride spray (Gustafsson and Sandin, 2009), which adds a significant dynamic mechanical component to the stimulus. The degree of cooling as well as the dynamic component may vary significantly depending on the technique used for application and is difficult to reproduce with accuracy across laboratories. The standard method of measuring sensitivity to thermal stimuli in rodents is the radiant heat paw withdrawal method (Hargreaves *et al*., 1988). The assay is sensitive to factors like posture, exact focus of the beam and the adaptation temperature of the skin, which may be altered by the model itself, handling and procedures in the testing environment and by pharmacological effects of test compounds (Bennett and Xie, 1988; Luukko *et al*., 1994; Dirig *et al*., 1997; Le Bars *et al*., 2001). These factors may lead to systematic errors, and it is frequently unclear whether adequate controls are performed.

Regardless of model and readout, interpretation of behaviour is a challenge. The quality of the sensation eliciting an evoked response can only be inferred from the stimulus modality and characteristics of the response. It seems reasonable to assume that the reduced thresholds to von Frey stimulation seen in neuropathy and inflammation models reflects a reduced threshold to mechanical stimulation or some other sensory disturbance, but the relevance of this measure for pain or allodynia has been questioned (Ängeby-Möller *et al*., 1998; Le Bars *et al*., 2001; Bove, 2006). Using spontaneous motor activity and gait analysis as a measure of pain may have utility in arthritis models but not in neuropathy, indicating that in the latter condition, these parameters reflect motor rather than sensory abnormalities (Ängeby-Möller *et al*., 2008; Piesla *et al*., 2009; Matson *et al*., 2010; Mogil *et al*., 2010). In pharmacological studies, most, if not all, behavioural readouts may be confounded by on-target and off-target effects unrelated to analgesia. Confounding effects

of standard benchmarking compounds like NSAIDs, antiepileptics and opiates are well known, if not always considered in experimental design and interpretation of results, but the problem is greater with novel compounds, where lack of precedence makes adequate control imperative.

How well do the models correspond to clinical pain?

The common models are best viewed as standardized, simplified procedures designed to reflect functionally significant mechanisms of chronic pain. For practical and ethical reasons, the models are usually of relatively short duration while clinical subjects have frequently suffered pain for years, which may have consequences in several domains not represented in the animal models. Models are often based on evoked responses and are usually used with single dosing of test compound. These factors impose some limitations in the modelling and need to be considered when data are interpreted.

Chronic pain related to osteoarthritis is typically activity-related but in more advanced disease also present at rest (Hunter *et al.*, 2008). Quantitative sensory testing has revealed increased sensitivity to stimuli, particularly pressure to the painful area, which disappears together with spontaneous pain after joint replacement (Ordeberg, 2004). As described previously, both gait analysis and evoked responses are used in animal arthritis models as behavioural correlates to the clinical signs and symptoms. The most commonly used carrageenan- and FCA-induced arthritis models are relatively acute (testing usually performed within a week of induction) and not particularly disease like in terms of pathophysiology.

Animal studies on peripheral neuropathy are dominated by traumatic lesions, unlike clinical trials where painful diabetic neuropathy or postherpetic neuralgia is the rule (Rice *et al.*, 2008). In several other aspects, modelling of neuropathic pain diverges from the clinical situation. Animals are usually tested within a few weeks of the insult when inflammation may be a confounding factor. As regards signs and symptoms, human neuropathic pain is characterized by spontaneous pain associated with positive and negative somatosensory signs. In a recent comprehensive study of 1236 patients with neuropathic pain of diverse, mostly peripheral, origin, patients across all groups reported high levels of ongoing pain and showed various degrees of sensory loss, mainly in the non-nociceptive domain, and gain of function, predominantly in nociceptive parameters, when investigated with quantitative sensory testing (Maier *et al.*, 2010). In this clinical material, less than 20% of the patients showed changes in cold pain threshold compatible with increase sensitivity. Equivalent figures were 24% for heat pain threshold, 20% for dynamic mechanical allodynia, 36% for blunt pressure and 29% for pin prick.

Thus, ongoing pain is the predominant complaint of the patients and the most common primary outcome variable of clinical trials. As mentioned in the discussion of the different neuropathy models, they seem to feature components of pain or discomfort, but these behaviours are rarely quantified. Instead, the models are almost exclusively reliant on measuring sensory hyperphenomena. There is, however, a growing interest in developing alternative approaches to measure ongoing pain, subjective components and disability in both inflammatory and neuropathic models. In veterinary practice, interpreting behaviour is necessary to understand whether an animal is in pain or responding to treatment. Similar approaches are being tried in experimental work. An ethogram for systematic behavioural assessment has been developed to quantify pain and analgesia in the rat, originally in conjunction with recovery from surgery (Roughan and Flecknell, 2004). A recent study identified and characterized facial expressions in mice interpreted to indicate pain and possibly reflecting an emotional component (Langford *et al.*, 2010). Besides observational approaches, several laboratories have applied operant methods to quantify pain and analgesia (Tzschentke, 2007; Vierck *et al.*, 2008). Cain *et al.* (1997) used a delayed non-matching-to-position paradigm to demonstrate pain-related disability and therapeutic effect of morphine in a rat adjuvant arthritis model. Place conditioning paradigms have revealed a dissociation between sensory-discriminative and affective components on the formalin test (Johansen *et al.*, 2001) and provided evidence for the presence of ongoing pain in the SNL and SNI models (King *et al.*, 2009). Recently, a passive avoidance test in SNL rats showed that a mechanical stimulus causing a 'hyperalgesia-like' response facilitated avoidance learning whereas conventional von Frey stimulation did not (Wu *et al.*, 2010), extending earlier data demonstrating facilitated escape and avoidance behaviour in this model (LaBuda and Fuchs, 2000). Other emerging approaches include drug discrimination (Colpaert, 1999) and use of naturally occurring painful diseases such as feline idiopathic cystitis and canine osteoarthritis (Westropp and Buffington, 2002; Quessy, 2010). These approaches should enhance and expand our current battery of behavioural models, particularly by adding measures of ongoing pain and aversive components. Bridging the current gap between main clinical complaints and the parameters commonly measured in the animal models, particularly in neuropathy, may turn out to be one of the more important factors in enhancing prediction of analgesic modelling.

In the preclinical setting, drugs are usually tested as a single dose. In the clinic, drugs against neuropathic pain are titrated up in dose over days and weeks for tolerability and compliance reasons, and long-term exposure may be required for the full clinical efficacy of some drugs. Drug distribution may differ significantly depending on whether a compound is given as a single dose or repeatedly to achieve steady state. A single high dose may therefore lead to unexpected effects. Gabapentin may serve as an illustration. Its uptake from the gastrointestinal tract to the blood as well as from the blood to the cerebrospinal fluid is saturable (Stewart *et al.*, 1993; Luer *et al.*, 1999). After a single dose given orally or parenterally, a relatively small fraction of the drug enters the central nervous system (Welty *et al.*, 1993), which is the most likely site of action for analgesia. A minimum effective single dose in the rat yields plasma concentrations about three times higher than human maintenance dosing (Whiteside *et al.*, 2008). Although this difference is moderate, the higher concentration may allow recruitment of peripheral mechanisms of anti-nociception not or only minimally activated under normal clinical dosing conditions and may, at least hypothetically, be a reason why the compound is much more efficacious in models than in the clinic.

Prediction versus clinical effect of established and novel treatments

Animal models are usually optimized and validated with clinically used analgesics and sensitivity to an appropriate compound, for example, gabapentin for a neuropathic model or an NSAID for an arthritis model is then a prerequisite. Since these treatments are only moderately effective in the clinic, this validation principle is likely to introduce a positive bias in the modelling, by favouring specific analgesic mechanisms, models or readouts sensitive to drug effects that may even be unrelated to analgesia. Taking traumatic neuropathic pain as an example, a recent comprehensive meta-analysis of clinical trials indicated that only opiates reliably show analgesic efficacy, although the effect is variable and small on a population basis (Finnerup *et al.*, 2010). The figures are based on a limited number of studies and should not be over-interpreted, but it is nevertheless apparent that antidepressants and anticonvulsants have limited efficacy in these conditions. Reporting standards do not allow a proper meta-analysis of animal data, but in a systematic review of the literature, the majority of studies showed relatively consistent efficacy of these classes of compounds in the commonly used sciatic nerve lesion models (Kontinen and Meert, 2003). Similarly, NSAIDs have moderate analgesic efficacy in clinical trials of osteoarthritic pain (Laine *et al.*, 2008) but excellent efficacy in many inflammatory pain models.

If it had been possible to adjust the animal data for relevant tissue exposure of the different compounds, the picture might have been different. There is however a relative paucity of published pharmacokinetic-pharmacodynamic data comparing modelling and clinical conditions. An exception is a recent study that compares pharmacodynamic and pharmacokinetic data from published clinical trials with data obtained in rat experiments, for the most part performed at Wyeth (Whiteside *et al.*, 2008). All comparisons were based on minimally effective doses and single administration in the animal studies and maintenance doses in the human dataset, which is a limitation recognized by the authors. Under these conditions, effective plasma levels of celecoxib and indomethacine were only slightly higher in rat (efficacy model was FCA-induced inflammation with paw pressure readout) than in man. Treatments for neuropathic pain (SNL as efficacy model) showed a mixed picture where antidepressants were either ineffective or required 10–40 times higher plasma levels in rodents, while the anticonvulsants gabapentin, lamotrigine and carbamazepine were active at concentrations one to three times the human levels. The data suggest that the animal models as used in this study were able to predict efficacy of some but not all mechanisms addressed by the drugs. It is interesting that tricyclic antidepressants, which constitute the overall most efficacious drug class for neuropathic pain in the clinic (Finnerup *et al.*, 2010), came out poorly in the rat model, but as mentioned earlier, neither anticonvulsants nor tricyclic antidepressants are particularly effective in neuropathic pain of traumatic origin.

Lack of efficacy has been estimated to be the cause of about 30% of failures in clinical phase of drug development (Kola and Landis, 2004), and several potential analgesics have failed in clinical trials over the last two decades. The tachykinin NK1 (substance P) receptor was supported by a large body of preclinical evidence (Henry, 1993), but antagonists have shown little (Dionne *et al.*, 1998) or no efficacy (Hill, 2000) in clinical studies. In terms of animal modelling, the NK1 receptor has been a particularly challenging target due to species differences in receptor affinity, which made use of the established rodent models problematic. Some antagonists had significant off-target activity when used in rodents, and compounds tested in relevant species showed variable efficacy (Berge and Ståhlberg, 1993; Karlsson *et al.*, 1994; Urban and Fox, 2000). A problem in evaluating the role of animal modelling in this case is that a fair amount of the data generated was only published as abstracts and in proceedings. Animal data from my own company (Astra Pain Control) that lead to loss of confidence in NK1 as a target for analgesia were never published.

Another unsuccessful approach was the combination of morphine and the *N*-methyl-D-aspartate (NMDA) receptor antagonist dextromethorphan (MorphiDex) predicted from animal studies to have improved efficacy and reduced tolerance development compared with morphine alone. These claims were not supported in clinical studies; in fact, the low level of tolerance development seen in these and other clinical studies compared with the rapid and total tolerance development characteristic for rodents exposed to opiates challenges the face and construct validity of rodent models for this purpose (Galer *et al.*, 2005). Making exact predictions from rat to man as regards opiate effects is further complicated by significant species differences in drug metabolism (Handal *et al.*, 2007).

Among other drug candidates that failed in clinical trials and human experimental pain models in spite of promising animal data are the sodium channel blocker 4030W92 (Trezise *et al.*, 1998; Wallace *et al.*, 2002; 2004; Liu *et al.*, 2003) and the COX-2 inhibitor GW406381 when tested in postherpetic neuralgia (Shackelford *et al.*, 2009) and, more surprisingly, in osteoarthritis (Boswell *et al.*, 2008). The latter compound did, however, show efficacy in human acute postoperative pain (Varner *et al.*, 2009) and migraine (Wentz *et al.*, 2008).

Modelling has been more successful in other cases. The introduction of spinal opiate administration for analgesic therapy was based on studies in rodents using simple nociceptive assays (Yaksh and Rudy, 1976; Onofrio *et al.*, 1981; Bennett *et al.*, 2000). Other examples are the COX-2 inhibitors (Rao and Knaus, 2008), pregabalin (Horga de la Parte and Horga, 2006; Kavoussi, 2006) and recently tapentadol (Prommer, 2010) and anti-nerve growth factor treatment for osteoarthritic pain (Cattaneo, 2010, Watson *et al.*, 2008).

It appears that the traditional animal models used appropriately may predict analgesic efficacy, but it is evident that a positive outcome in animal studies does not guarantee success in the clinic. The examples indicate that the degree of success is independent of the novelty of the target pathway promoted.

Way forward

Quality of prediction can be improved by establishing a quantitative relationship between drug exposure in relevant tissue, target engagement and behavioural readout, or in other words, by evidence that the effect observed is obtained by the intended receptor interaction. Off-target effects not

recognized or on-target effects in tissues and systems unrelated to the intended mode of action can be major confounding factors. In animal studies, determination of drug exposure in relevant tissues is usually feasible. Other tools should be used to strengthen the understanding of pharmacokinetic–pharmacodynamic relationships, including biochemical biomarkers, which may be harder to find in the analgesia field than in some other areas of drug research, electrophysiology, as well as functional histochemistry and imaging. There is growing awareness in the scientific community that predictive pharmacokinetic–pharmacodynamic modelling requires a set of biomarkers to establish a consistent chain of events from target–drug interaction to clinical effect (Danhof *et al.*, 2005).

The other side of the coin is the clinical situation. There is substantial interest in developing tools and algorithms for mechanism-based patient segmentation in neuropathic pain by means of sensory profiling and symptoms (Attal *et al.*, 2008; Maier *et al.*, 2010). The underlying pathophysiology is still incompletely understood, which is a limiting factor in determining construct validity of animal models and ultimately their ability to facilitate accurate prediction of clinical efficacy. Strategies to circumvent the gap in our understanding of clinical pathophysiology include development of more disease-like rodent models for conditions like painful chemotherapy-induced neuropathies (Authier *et al.*, 2009), musculoskeletal pain (Bove *et al.*, 2009) and bone cancer pain (Jimenez-Andrade *et al.*, 2010), use of animals with natural painful diseases (Quessy, 2010) or application of more direct measures of pain by utilizing operant methods (Vierck *et al.*, 2008). Regardless of advances in methodology, validation needs to focus not only on the phenomenological similarity with the clinical condition but even on molecularly and physiologically relevant mechanisms.

As we have seen, published data based on animal models tend to present an optimistic view on efficacy, although this may in many cases be due to poorly controlled drug distribution and exposure. It is, however, reasonable to assume that the literature is unbalanced in that negative data are less likely to be published. Material transfer agreements, particularly between companies, but even between companies and academic scientists, may prevent publication of unfavourable data. Academics may simply not find it worthwhile to prepare a manuscript with negative data, given the common perception that publication will be an uphill battle. Depending on the policy of the company and the enthusiasm of the scientists, programme closure may or may not be followed up in publication of data that would explain whether a program was stopped for tactical reasons, for lack of efficacy in animal models or because of other factors. By the end of the day, valuable data may not be available to the scientific community.

Another obstacle to scientific progress is variable methodological quality and inadequate reporting standards. A systematic review of experimental design, statistical analysis and reporting in 271 biomedical publications involving laboratory animals was recently published (Kilkenny *et al.*, 2009). The authors found that randomization and blinding procedures were reported in less than 15% of the studies. Only 59% stated the hypothesis or objective of the study and the number and characteristics of the animals used. Of the publications that used statistical methods, 70% described their methods and presented the results with a measure of error or variability. The situation is similar for reporting in the analgesia field (Rice *et al.*, 2008). Uncertainty about blinding and randomization is a serious concern; uncontrolled experimental bias has been suggested to be a major problem in interpretation of drug effects and even a factor contributing to clinical attrition (Lindner *et al.*, 2003; Eisenach and Lindner, 2004; Lindner, 2007).

Recently, a set of guidelines (ARRIVE – Animals in Research: Reporting *In Vivo* Experiments) were published in several high-profile journals, including the *British Journal of Pharmacology*, with the aim to improve the standard of bioscience research reporting (McGrath *et al.*, 2010; Kilkenny *et al.*, 2010a, b). The guidelines provide a checklist of information that should be addressed in scientific papers and should be useful for scientists preparing and presenting a study as well as for editors and referees. An on-line 'extended methods form' has been proposed with details on methodological aspects specifically relevant to analgesia research (Rice *et al.*, 2008). These guidelines are to some extent complementary, but both emphasize blinding and randomization procedures, adequate statistics with explanation for lost data and in-depth description of methodological factors.

Conclusions

The available animal models for analgesia constitute a great toolbox, but the track record for prediction is truly mixed. Several areas have been identified where there is opportunity for improvement: experimental design and reporting, novel models and readouts, emphasis on pharmacokinetic–pharmacodynamic relationships, to mention some of the more important. But regardless of advances in design and methods, the main challenge in predicting efficacy will still be to identify and use models that reflect critical pathophysiological processes in a complex target patient population.

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