Ongoing research strongly suggests that evidence of concentration-effect relationships is necessary for translational purposes. Given the lack of specific biomarkers of disease, assessment of target engagement is fundamental for characterising the pharmacokinetic-pharmacodynamic properties of novel compounds and interpreting response across species.

Translation of drug effects from experimental models of neuropathic pain and analgesia to humans

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Neuropathic pain research remains a challenging undertaking owing to: (i) the lack of understanding about the underlying disease processes; and (ii) poor predictive validity of the current models of evoked pain used for the screening of novel compounds. Common consensus is that experimental models replicate symptoms (i.e. have face validity but no construct validity). Another issue that requires attention is the sensitivity of endpoints to discriminate drug effects that are relevant to the disease in humans. In this paper we provide an overview of the pre-clinical models that can be used in conjunction with a model-based approach to facilitate the prediction of drug effects in humans. Our review strongly suggests that evidence of the concentration–effect relationship is necessary for translational purposes.

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Introduction

Background

Physiological pain usually arises as a result of the activation of nociceptive afferents by noxious stimuli that can lead to tissue damage (e.g. during inflammation). Pain can also occur in the absence of or by sub-threshold stimulation of peripheral nociceptors. This type of pain is called neuropathic pain (NP). The International Association of the Study of Pain (IASP) defines NP as pain arising as a direct consequence of a lesion or disease affecting the somatosensory system [1]. Following tissue injury, pro-nociceptive mediators are released that sensitise peripheral nerve terminals, which in turn trigger neurochemical and phenotypic alterations of the sensory nerves and increased excitability of spinal dorsal horn neurons (central sensitisation) [2]. Common causes of nerve damage and subsequent pain response include metabolic diseases, infection, ischemia, traumatic injury, malignancy, adverse drug reaction and toxins. However, the cause of NP symptoms might not be easily linked to a cause in the majority of patients experiencing it [3]. The lack of a clear aetiology has major consequences for the selection of treatments. In addition, it underlies some of the main hurdles in target identification and compound screening in drug discovery. Current efforts in R&D therefore predominantly focus on the suppression of symptoms, rather than on the treatment of the primary cause(s) of pain [4]. In addition, these drugs

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often produce unacceptable side effects, making the clinical management of pain syndromes a major challenge [5].

The hallmark of NP is impaired sensation, with sensory dysfunction manifesting itself as hypo- or hyper-aesthesia for one or more modalities [6]. As a matter of fact, heightened pain perception to harmful painful stimuli (hyperalgesia) or to normally painless stimuli (allodynia) is often accompanied by spontaneous pains such as burning or electric-shock-like pain, paroxysms and dysaesthesia [7,8]. Clinically, symptoms are classified as positive or negative. This classification, which was primarily developed for diagnostic purposes, has also driven the choice of endpoints in clinical research protocols. Positive symptoms include mechanical and thermal allodynia and hyperalgesia, as well as temporal and spatial summation, whereas negative symptoms are indicative of loss of sensory and motor function [9,10].

Despite the symptomatic nature of drug therapy, existing treatments are suboptimal, with effective reduction in pain by an average of 40–50% from baseline in only 30–40% of patients [4,11]. Available treatment options include opioids, anticonvulsants and antidepressants, all of which have a direct or indirect suppressant effect on neuronal activity. Besides limited efficacy, these compounds often cause dose-limiting toxicities that, along with impairment in the quality of life, prevent titration to effective dose ranges [4]. From a drug discovery perspective, these findings are associated with a high attrition rate in the progression of candidate molecules. Although the overall probability of a candidate in development being approved is ~11%, the figure for NP candidates is as low as 3–5% [12].

Screening and development of analgesic drugs

From the above information, it is evident that challenges exist not only in the identification of suitable targets but also in predicting a priori the efficacy of compounds in humans. Given the gaps in the understanding of the mechanisms underlying NP disorders, drugs are tested pre-clinically without any evidence as to which target will yield a clinically relevant response [11]. This is further reinforced by the paradigm currently used for the screening of compounds, which relies on evoked-pain response associated with general positive symptoms such as allodynia and hyperalgesia. These models enable characterisation of the behavioural expression of pain processing, rather than specific features of the mechanisms of hypersensitisation [12,13].

Advances in this field require a clear distinction between the role of target, endpoints and drug properties as primary causes of attrition. In this article we will explore the value of pharmacokinetic–pharmacodynamic (PKPD) modelling in conjunction with pre-clinical models of NP as a tool for the translation of pre-clinical findings. This concept has been previously illustrated for the evaluation of the anti-inflammatory and antihyperalgesic effects of different cyclooxygenase (COX) inhibitors [13,14] (Fig. 1).

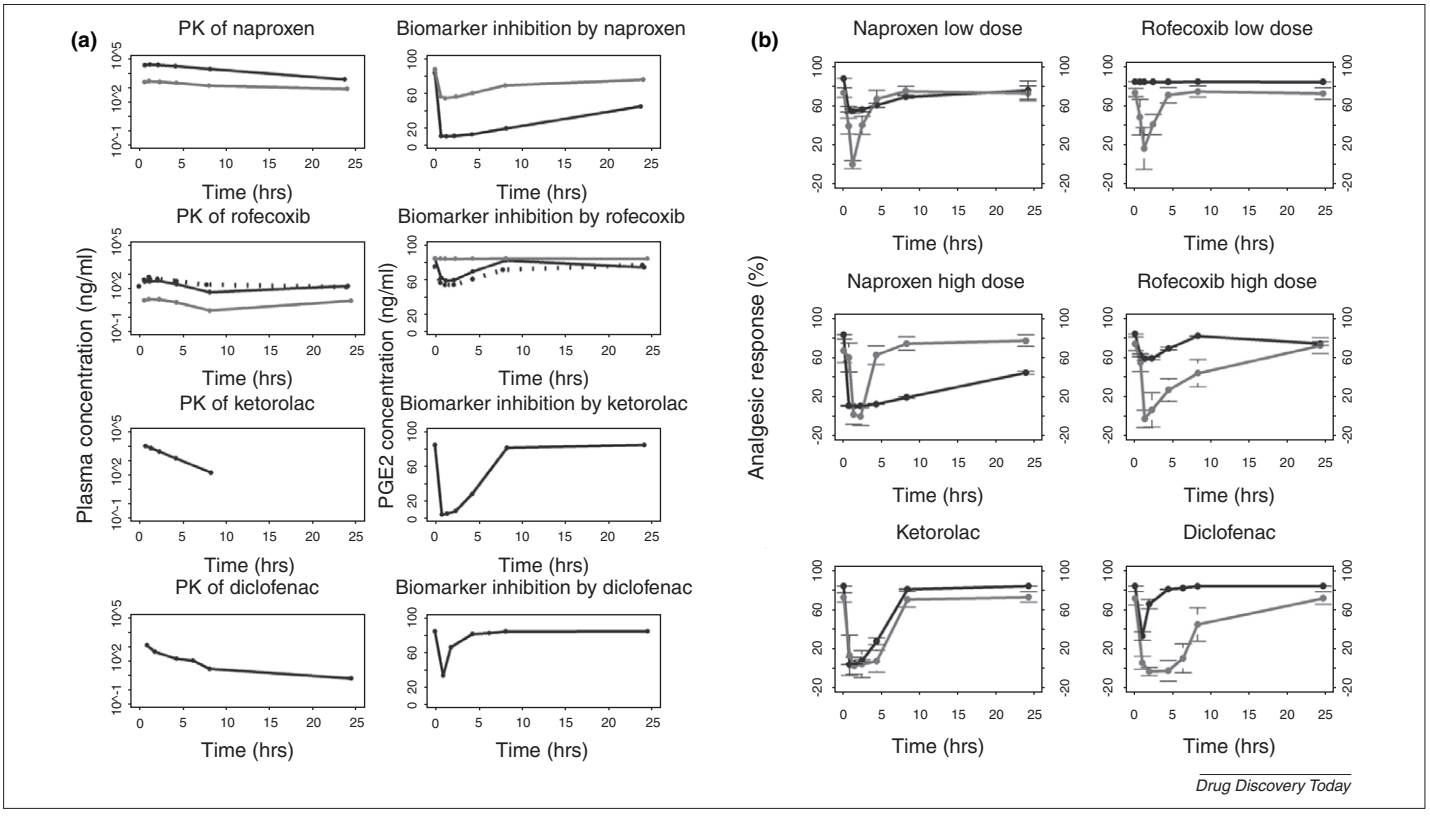

FIGURE 1

(a) Simulated concentration time course and corresponding PGE_2 profiles following COX-inhibition by naproxen, rofecoxib, ketorolac and diclofenac. Panels with more than one profile depict experiments in which different dose levels were tested. **(b)** time course of the predicted PGE_2 levels (black line) and corresponding analgesic response profiles (grey line) in the FCA model of inflammatory pain. PKPD modelling of the anti-hyperalgesic effect shows that changes in paw pressure withdrawal threshold are non-linearly correlated with PGE2 inhibition. Such a non-linearity between pharmacological effects and behavioural measures must be considered when defining the dose rationale in humans. Data are presented as mean ± standard deviation.
Modified, with permission, from Ref. [13].

Likewise, we envisage the use of model-based estimates of potency and efficacy as the basis for the dose rationale and improved prediction of efficacy in humans. Such an approach enables discrimination of drug-related factors from other causes of attrition in early drug development.

Animal models and pain signalling and processing

In this review, the classification and underlying pathophysiology of pain are given in terms of the various experimental models of evoked-pain (Fig. 2) and common behavioural endpoints [15]. To this end, we refer to the mechanisms of pain signalling and processing, which have been described as a continuum with three phases, specifically from nociceptive (phase 1) to inflammatory (phase 2) and finally neuropathic (phase 3) pain. A correlation between the pain phases and the various models and measures is given below as a basis for further discussions on the choices of parameters for PKPD models of pain.

The first phase within this continuum involves transient nociception and results from a noxious stimulus. Thus, at this stage there is minimal inflammatory response. Animal models of acute pain measure behavioural responses of naïve animals to noxious stimuli. Transmission of the stimulus occurs across A-δ and C fibres, which propagate fast and slow nociceptive responses, respectively [16]. Noxious heat is the most common stimulus applied in these models whereby the analgesic effects of drugs can be measured [17].

Phase 2 pain or inflammatory pain results from tissue damage and inflammation secondary to a noxious stimulus. In this phase, there is input from the damaged fibres to the central nervous system (CNS). Tissue injury causes mediator release creating an 'inflammatory soup', which upregulates or activates nociceptive afferents. This barrage of information supplied to the spinal cord triggers hyperresponsiveness of dorsal horn neurons [18]. A consequence of this hyperactivity is the development of symptoms such as hyperalgesia (an increased response to noxious stimuli) and allodynia (a painful sensation to non-noxious stimuli). Hyperalgesia and allodynia can be primary (i.e. close to the damaged area) or secondary (i.e. in tissues away from the damaged area). Secondary occurrence of these symptoms is indicative of central (spinal cord) sensitisation [16]. The neural pathways for transmission of this type of pain are similar to those for nociceptive pain. However, the spinal excitability is caused by release of inflammatory neuropeptides from the activated C type primary afferents. Animal models that involve application of an inflammatory agent [formalin, capsaicin, mustard oil, carrageenan or Freund's complete adjuvant (FCA)] elicit hyperalgesia and allodynia, thus mimicking phase 2 pain [16].

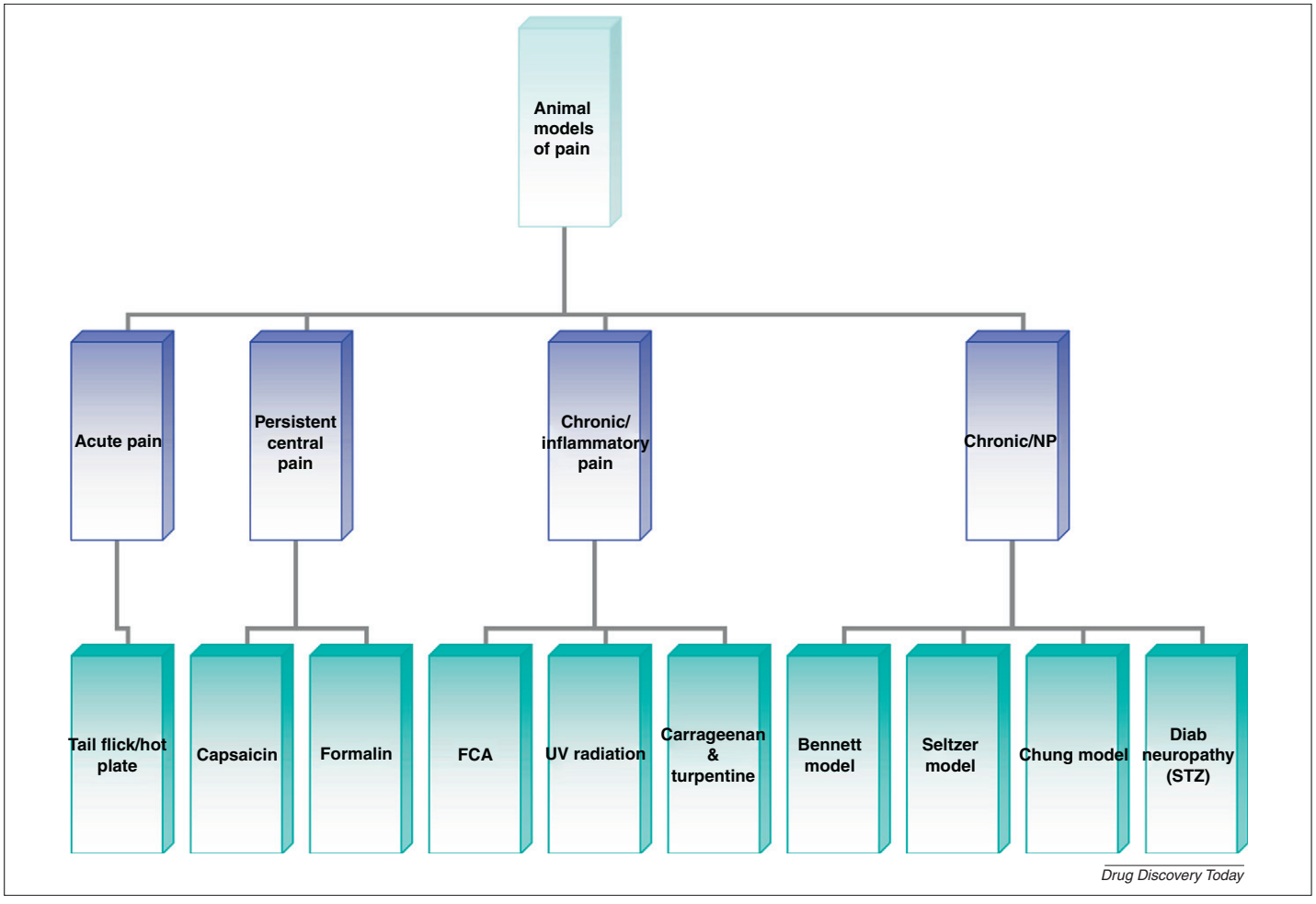

FIGURE 2

Overview of commonly used experimental pain models for the screening of novel molecules in drug discovery and early characterisation of the pharmacological properties in drug development.

Phase 3 or NP arises from lesions to or dysfunction of the nervous system, and thus all or part of the afferent input comes from damaged neurons. It manifests as spontaneous pain (stimulus-independent pain) as well as secondary hyperalgesia and allodynia [16,18]. Animal models of NP in which phase 3 pain is elicited include partial or complete denervation of the sciatic nerve or of the spinal L5/6 nerves. In fact, partial denervation methods are widely used in NP research owing to their similarity to human NP. The Bennett, Seltzer and the Chung models are some of the common animal models used to describe this phenomenon [16].

Although NP can be due to different aetiologies, all causes lead to damage of the nociceptive pathway [19]. However, it is important to realise that the assessment of pain in animal experiments remains essentially an indirect measure. There is no way to characterise the quality (shooting, stabbing, lancing) or intensity of pain. With the exception of aversive behaviour to potentially noxious stimuli (which includes vocalisation, biting, licking, shaking of affected limb), all measurements are based on a nocifensive reflex [16]. These models are potential candidates for surrogacy of human evoked pain, but are inadequate models of spontaneous pain which is frequently observed in patients [7]. Other endpoints such as latency of hind-paw withdrawal or intensity of the pressure producing withdrawal are applied as measures of treatment effect in acute and chronic pain states irrespectively of underlying differences in pathophysiology [16]. The von Frey test is based on the assumption that a pain threshold can be reached, which reflects central hypersensitisation. However, in this test, low threshold mechanoceptors and nociceptors are activated, indicating the stimulus is non-specific [17].

Specificity of pain response and endpoints in pre-clinical models

The endpoints used in experimental models of pain can be categorised into (i) pain-related behaviour, such as paw licking or (ii) threshold response, such as the latency time to paw withdrawal [20]. The behaviour considered most indicative of pain in animals includes autotomy or self-attack (assessed by counting the number of wounds inflicted), hyperalgesia (a strong withdrawal response to a moderate heat stimulus) and allodynia (withdrawal in response to non-noxious tactile or cold stimuli) [21]. These measures of pain perception involve more than just nociceptive pathways. They require the contribution of various CNS structures, which can be indirectly or directly susceptible to primary or secondary effects of drugs and can differ considerably from their counterparts in humans. On the other hand, standard evaluation methods such as the hot plate or tail flick tests assess the presence of pain-like behaviour, but they provide little information on the nature or quality of ongoing pain. Furthermore, known differences exist in the biological substrate and natural course of disease associated with endpoints in different models of NP (Fig. 3). For example, dynamic, brush-evoked allodynia in humans is mediated by low-threshold fibre input, whereas allodynia in rats is static in nature and mediated by high-threshold fibre input [22]. The tail-flick method, capsaicin- or formalin-induced pain model, the Bennett or Seltzer NP models do not have a human equivalent [23].

Similar considerations apply to the repertoire of behavioural changes that are indicative of spontaneous pain (e.g. increased weight bearing on the uninjured hind limb, guarding behaviour of the injured paw and licking of the injured paw coupled with 'gentle' biting and pulling of toe nails) [22]. In fact, endpoints classified as representative of spontaneous pain in humans have also been questioned regarding the extent of their predictive validity. Yet, although pain-like behaviour in animal models of NP is common, the corresponding symptoms are relatively infrequent in patients [24].

Unfortunately, understanding of the correlation between the mechanisms of pain response (overt behaviour) and the underlying pathophysiology (pain signalling and processing) remains poor, especially if one considers the limited data on target site exposure or other downstream effects of drug action collected in experimental models of NP (Fig. 4). This gap prevents the use of a mechanistic approach for extrapolation of drug effect on behaviour into accurate estimates of analgesia in patients. Furthermore, pain behaviour in humans is more varied and heterogeneous. In contrast to the pre-clinical measures, the representation of clinical characteristics of NP has been based on (i) spontaneous, ongoing pain, with clear reference to pain quality and sensory loss which includes varying degrees of hypoaesthesia and (ii) evoked pain, manifested by hyperalgesia and allodynia (Table 1) [25]. Hence, several visual and numerical rating scales, such as the McGill pain questionnaire, have been developed for the assessment of pain in clinical studies that capture the various dimensions of the disease [26]. These scales provide information about the intensity, duration and location of the pain [27].

Lastly, it should be noted that it may not be possible to reproduce integral components of pain such as the cognitive-affective part in pre-clinical species. The differentiation between response features (e.g. pain relief and worsening) and mood disturbances in human pain cannot be replicated [22,28]. This limitation is particularly important in chronic pain conditions such as NP. Despite seemingly pathophysiological similarities, none of the current models of neuropathy relates directly to clinical NP conditions [23,29].

Predictive value of pre-clinical models

Given the aforementioned differences across species, questions arise regarding the translational and predictive ability of pre-clinical models to describe the clinical phenomenon. Animal models are based exclusively on 'pain-like' behaviours or facilitated withdrawal reflexes which, in turn, are relatively infrequent in humans. By contrast, the most common human complaints are tingling, paresthaesia and numbness rather than pain [24]. Therefore, the terms nociception and antinociception are preferred instead of pain and analgesia [16]. It should also be unequivocal that animal models reproduce overt symptoms (i.e. they have face validity but are contextual in nature). For instance, paw withdrawal in response to a heat stimulus is observed with inflammatory pain as well as NP [30].

From a scientific perspective, the prerequisites for predictive value in experimental research include appropriate face and construct validity. An experimental model of disease or injury should be able to reproduce the symptoms observed in the clinical condition (face validity). At the same time, it is essential to mimic the pathophysiological changes that cause the overt symptoms (construct validity) [13,31]. Commonly used animal models of NP, such as the chronic constrictive injury (CCI) or sciatic nerve

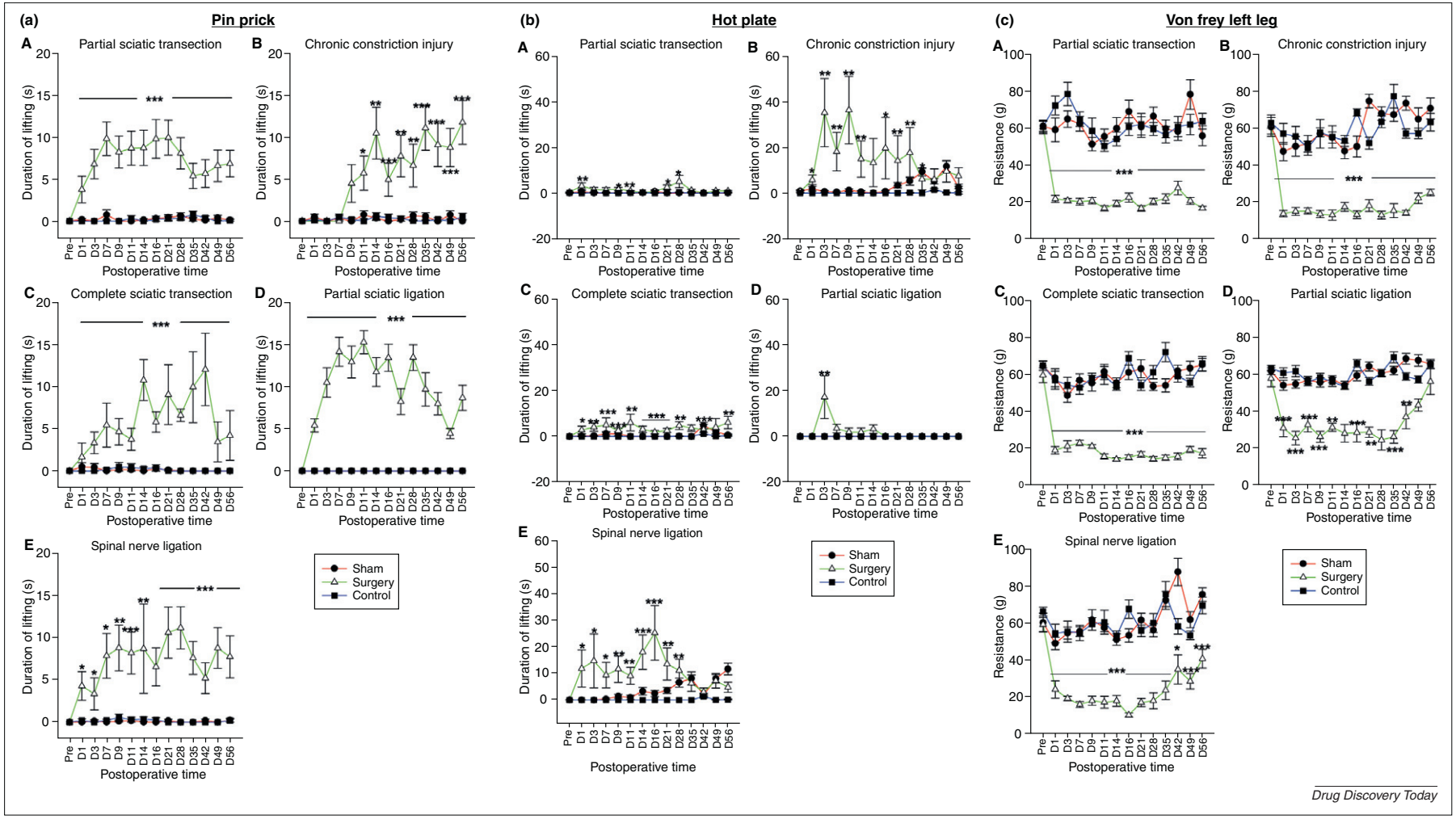

FIGURE 3

Behavioural measures of pain in five different models of nerve injury. The ability to discriminate drug effects does not solely depend upon the choice of experimental model but also on the choice of the endpoint for pain response. As can be seen in the profiles above considerable differences exist in the duration of lifting of the left hind paw after stimulation with a pinprick **(a)**, hotplate **(b)** and von Frey hair **(c)**. These differences also vary over time, indicating the progression of an undergoing disease process. Given are mean (SEM) values of control, sham operated and surgery groups before and at several time points up to 56 days after surgery. Reprinted with permission from [32].

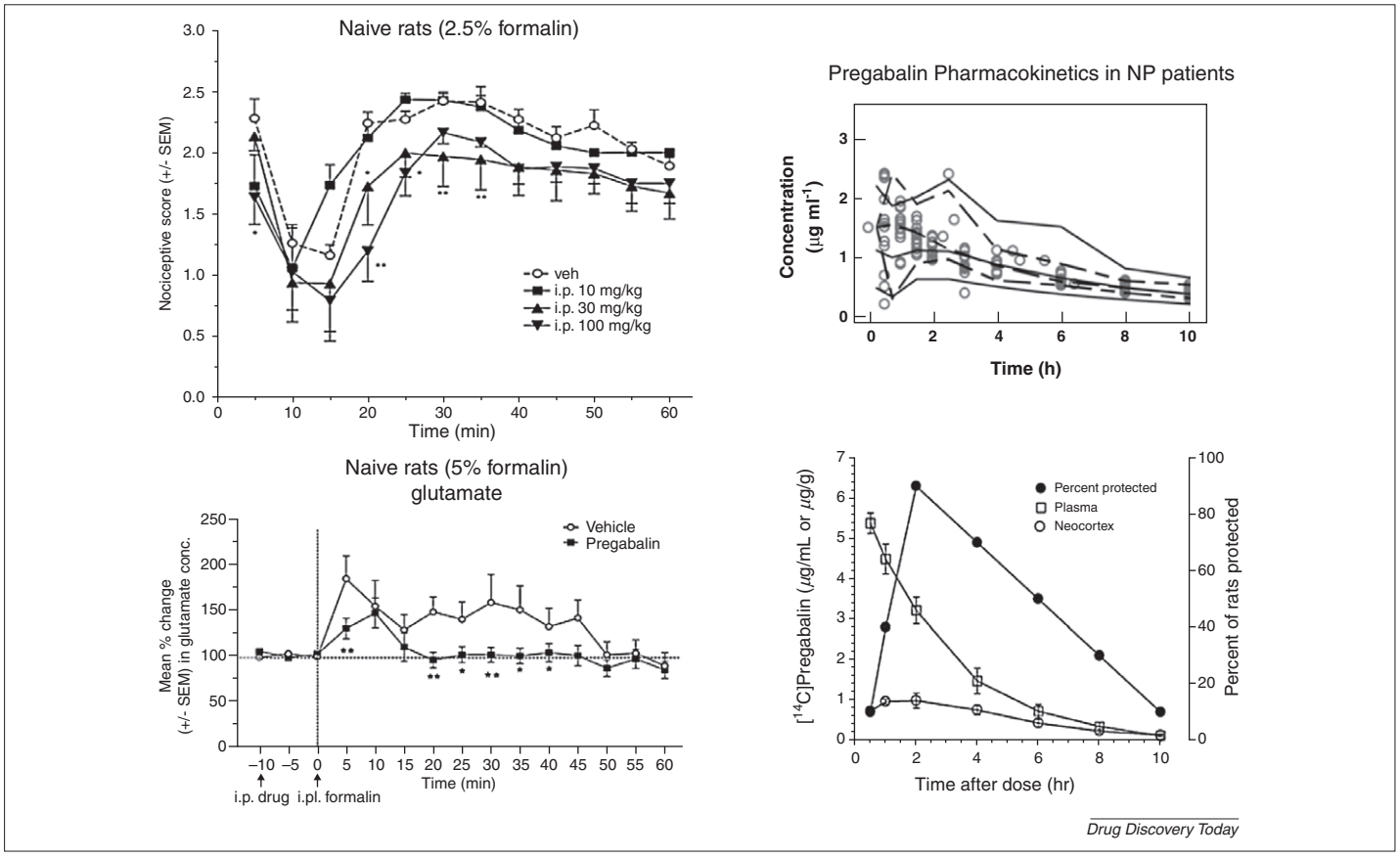

FIGURE 4

Despite evidence of peripheral hypersensitisation in the formalin-induced pain model, drug effects on nociceptive response are short-lasting relative to the underlying pathophysiological changes, as indicated by the differences in the time course of pain behaviour (upper left panel) and concentrations of glutamate (lower left panel) in the spinal dorsal horn after administration of pregabalin [59]. Availability of pharmacokinetic and pharmacodynamic data is essential to explore such discrepancies in a quantitative manner and subsequently define a suitable dose range in humans. In this specific example, some discrepancy is observed between drug effects in animals and humans. Response to pregabalin in rats occurs at levels above 4.0 μg/ml (lower right panel), whereas therapeutic levels after administration of a 50 mg dose are below 2.0 μg/ml. Reprinted with permission from [59,82,83].

ligation (SNL), replicate elements of the pathology seen in clinical pain states but have questionable construct validity with respect to specific pain syndromes, such as post-herpetic neuralgia (PHTN) or diabetic neuralgia (DN) [24,32]. In addition, homogenous strains are used that diverge from the heterogeneity of disease processes and progression in patients [33]. Such an optimisation of experimental conditions can work against current efforts to predict treatment response in humans [12].

The failure in the development of neurokinin (NK)1 antagonists as a putative mechanism for the treatment of NP illustrates the challenges in translational pharmacology research. The purported action of these compounds was blocking the actions of substance P, which has an important role in mammalian nociception. Lee *et al.* demonstrated that NK1 antagonists (intraplantar or intrathecal) attenuate NP symptoms in the SNT model. Based on their experiments, it was suggested that pain control methods targeting substance P and calcitonin gene related peptide (CGRP) should be designed as new therapeutic strategies for peripheral NP [34]. However, in clinical studies, NK1 antagonists failed to reproduce the promise observed in animals [35]. In this example, the pathway selected had little relevance to the clinical disease, and the chosen endpoint did not differentiate this. Unfortunately, despite documented failures, some authors continue to defend the predictive validity of current animal models [22].

Target validation and new interventions

The absence of a mechanism-based classification of pain syndromes consequently affects the selection of appropriate targets [36]. These hurdles are augmented by the lack of a process that ensures an integrated assessment of drug properties from target selection to proof-of-concept [11,14]. In the subsequent sections of this paper, we discuss the advantages of establishing PKPD relationships as the basis for the prediction of efficacy in humans. Our objective is to show how quantitative pharmacology techniques can assist drug discovery in distinguishing whether the high rate of false-positive findings in early drug development is caused by a lack of construct validity of animal models or simply because of the poor sensitivity of evoked response endpoints, which consequently leads to inaccurate estimates of the dose required for the evaluation of efficacy in humans. PKPD relationships themselves will not eliminate the issues with construct validity or unsuitability of behavioural endpoints. We state, however, that PKPD relationships can be extremely useful if used as a biomarker of pharmacology, in which properties such as binding, target

TABLE 1

Proposed relationship between neuropathic pain mechanisms, clinical symptoms and signs and known targets in current therapeutic interventions

Compound/intervention	Targets	Neuronal processes/pathophysiology	Symptoms/signs
Lidocaine, Carbamazepine, Lamotrigine	Na-channels	Pheripheral nociceptor hyperexcitability Ectopic impulse generation, oscillations in DRG	Spontaneous pain
Capsaicin	TRPV1-receptor TRPM-receptor TRPA1-receptor ASTC-receptors	Peripheral nociceptor sensitisation Reduced activation threshold to: Heat Cold Mechanical stimuli	Heat hyperalgesia Cold hyperalgesia Static mechanical allodynia
Sympathetic blocks	α-receptor	noradrenaline	
Opioids	Presynaptic μ-receptors	Central dorsal horn hyperxcitability	
Gabapentin, Pregabalin, Ziconotide	Ca-channels Post-synaptic: NMDA-receptors NK-1 receptors Na-channels Intracellular cascade	Central sensitisation on spinal level Ongoing C-input induces increased synaptic transmission	Spontaneous pain
Ketamine	GABA-B receptors	Amplification of C-fibre input Intraspinal inhibitory interneurons (functional degeneration)	
Baclofen	Glycine-receptors	GABA-ergic Glycine-ergic	Dynamic and punctuate mechanical allodynia
Antidepressants **Amitriptyline** **Venlafaxin** **Duloxetine**	α1-receptors 5-HT-receptors	Changes of supraspinal descending modulation	
Clonidine	α2-receptors	Inhibitory control (NA, 5-HT)	

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); ASTC; GABA, γ-aminobutyric acid; NK1, neurokinin1; NMDA, *N*-methyl-D-aspartate; TRPA1, transient receptor potential cation channel, subfamily A, member 1; TRPM, transient receptor potential channel melastatin; TRPV1, transient receptor potential cation channel subfamily V member 1 (also known as capsaicin or vanilloid receptor). Table adapted, with permission, from Gilron and Coderre [4,79].

activation or blockade and subsequent downstream effects are characterised in a quantitative manner.

Translational pharmacology

The high attrition rate in the development of compounds for NP has prompted many investigators to reinvestigate how pre-clinical findings correlate with efficacy data in patients [22,24,33,37]. These reviews share a common view that evoked pain responses are inadequate measures of pain behaviour and do not represent pain processing in humans. They propose solutions ranging from modifications of current experimental models to the use of engineered mutant models [33,38]. However, drug effects are often parameterised in terms of ED_{50}, without accounting for the differences in PK across species and the requirements for characterisation of a dose–response curve [39]. Finally, the duration of the effect and its relationship to target engagement is completely set aside [40].

Among the various publications, it is worth mentioning the attempt of Whiteside and Kennedy to explore the correlations between pre-clinical and clinical data by comparing drug exposure in rats at the minimum effective dose (MED) for different models of inflammatory pain and NP with clinical exposure in patients at the maintenance dose levels. Their conclusion is that pre-clinical animal models are predictive of efficacious exposures in humans [41]. However, the authors seem to overlook the fact that efficacious exposure ranges were considerably higher in rats than in humans. They indicate that the MED in rats for a single dose is not representative of clinical exposure, nor of the effects observed after repeated dosing. Yet, total plasma concentrations are compared without taking into account interspecies differences in PK (i.e. drug distribution). For CNS drugs, the assumption that plasma concentrations reflect drug exposure at the site of action is often inappropriate. Another important aspect is the role of differences in metabolic rate, which lead to potential drug interactions if metabolites also have affinity for the target [42] (Table 1).

Overview of experimental pain models

As shown in Fig. 2, despite the differences in the aetiology of pain in common experimental models, the screening for NP compounds has also relied on the use of chronic inflammation models of pain [23]. In fact, the empiricism in the selection of compounds is further illustrated by the evaluation of anti-inflammatory drugs in NP models.

In the subsequent sections, we will explain which changes are required in the experimental design to enable appropriate characterisation of the concentration–effect curve for the screening of novel compounds and translation of findings across species.

Inflammatory pain

UVB model: acute cutaneous overexposure to UV radiation (UVR) causes thermal and mechanical hyperalgesia in rats and humans. Using a UVB source, the plantar surface of the rat hind paws is irradiated to cause inflammatory reaction. This injury induces a significant dose-dependent reduction in thermal and mechanical paw withdrawal thresholds, which peak 48 hours after irradiation. The inflammation is caused by apoptosis of epidermal cells induced by DNA damage [43]. Algogenic chemicals are also released following UVR inflammation. The cutaneous hypersensitivity results in thermal hyperalgesia as well as UVB mechanical allodynia. Spontaneous pain behaviour such as flinching, licking, excessive grooming or paw lifting are not seen with this model [44]. This model differs from the CFA model in that it does not produce an increase in the spinal basal c-Fos expression levels at the peak of sensory changes. There is little spontaneous activity in the primary nociceptors which, in turn, could sensitise the spinal nociceptive structure. This fact sets this model apart from the other algogenic models where central (spinal) sensitisation is observed. Hence, the UVB model is sensitive to study effects of peripherally acting analgesics such as NSAIDs [44].

Neuropathic pain

In NP models tissue injury is produced chemically or surgically, which leads to peripheral nociceptive sensitisation. These changes induce phenotypic alteration of sensory neurons and increased activation of spinal dorsal horn neurons. The development and maintenance of central sensitisation in the dorsal horn of the spinal cord ensues [18,45]. In persistent pain models an algogenic substance such as capsaicin or formalin is introduced subcutaneously or intraperitoneally [46]. More-specialised models envisage induction of nerve injury [47]. Although they differ by locus and type of injury, all produce behavioural insensitivity as a result of the trauma [48].

Formalin-induced pain model (FIP): the formalin test was developed for the screening of compounds with antinociceptive effects [46]. Recently it has been found to correlate with the CCI model, one of the best available models characterising NP behaviour [49]. Two phases of nociceptive behaviour are observed following formalin injection. The first phase starts immediately after injection and lasts for 3–5 min. It is caused by direct chemical stimulation of nociceptors and predominantly associated with activity in C fibres. Subsequently, there is a quiescent phase of 10–15 min. The second phase starts 15–20 min after the formalin injection and lasts for 20–40 min. Drugs effective against NP affect the onset and amplitude of the second phase (i.e. the quiescent phase is prolonged and the maximum intensity of pain is decreased). The effect of formalin is believed to result from central sensitisation of dorsal horn neurones, as a result of the initial barrage of inputs from C fibre nociceptor afferents during the first phase. A role for higher brain regions in maintaining this pain state has also been hypothesised [50]. The frequency of paw licking behaviour (PLB) per time interval is measured for NP drugs and compared with placebo [46].

Chronic constrictive injury models: in contrast to complete transaction of the sciatic nerve, which does not reflect NP pathophysiology in humans [51], these models either involve a loose ligature placed around the entire sciatic nerve (Benett model) or a tight ligature through half of the proximal sciatic nerve (Seltzer). Thermal and mechanical hypersensitivity is observed in both models, but because of experimental complexities, the procedure is difficult to reproduce leading to variability in evoked responses [52,53]. A variant to the CCI model was developed by Kim and Chung where the L5/6 spinal nerves are ligated and the L4 nerve remains intact. In this way the intact dermatomes of the paw can be tested, although the L4 nerve is at risk of damage owing to exposure [54].

Partial denervation models: these models were developed to simplify technical feasibility further, as well as to minimise variability associated with the degree of tissue damage. They enable the investigation of changes in injured primary sensory neurons and in neighbouring intact sensory neurons so that the relative contribution of both structures to the pathophysiology of NP symptoms can be investigated. The spared nerve injury (SNI) model involves axotomisation of the tibial and common peroneal nerves while sparing the sural nerve [47]. The tibial nerve transection (TNT) represents a further modification of the SNI model. These nerve injury models are considered representative of symptomatically induced pain, enabling the evaluation of three different components of NP *vis-a-vis* mechanical allodynia, cold allodynia and spontaneous pain. The threshold to response (withdrawal of the injured paw) is the primary measure for the assessment of drug effects [24,55].

PKPD modelling of pain response

Translational drug research requires the prediction in a strictly quantitative manner of the PKPD properties of drugs [56]. Assessment of PKPD relationships enables better understanding about how changes in drug exposure correlate to the pharmacological effects and overall response to treatment. To that purpose, experimental protocols must be designed and customised accordingly to ensure that appropriate pharmacokinetic and pharmacodynamic data are obtained. Among other things, one needs to consider which dose levels to use, how long to sample and whether systemic exposure reflects target site concentrations. These requirements contrast, however, with current practice for screening protocols, which are usually performed in a standardised '*one size fits all*' manner. As such, these experiments are less informative, often precluding accurate estimation of the parameters of interest.

Experimental requirements

Given that behavioural models are primarily used for the purposes of screening and ranking of compounds, it is crucial that potency estimates are accurate and expressed in terms of exposure (EC_{50}), rather than dose (ED_{50}). In this context, potency can be used as the basis for the scaling of PD estimates across species and/or endpoints. To that purpose, considerable changes to protocol design are required that involve modifications to the sampling procedures and the dosing rationale.

Experiments suitable for PKPD modelling must therefore consider some basic requirements regarding the time-course of response. For instance, a minimum number of samples per animal are necessary for reliable estimation of PD parameters (e.g. baseline, E_{max} and EC_{50}). This can be achieved by the use of sparse sampling and treatment with different dose levels. By contrast, PK sampling should provide enough information to characterise the absorption, distribution and elimination of the administered drug.

Although the ideal situation is to have PK and PD samples measured concurrently, this might not be feasible in behavioural models of pain. In this case, a PK experiment can be performed *prior to* the assessment of drug effects. The advantages of this approach have been elegantly demonstrated by Bender *et al.* [57,58] for the analysis of drug effects in the CCI animal model.

Another important point to consider is the observation window available for the evaluation of drug effects. Under non-stationary conditions, the disease process itself also needs to be characterised [59].

Data analysis requirements

PK and PD data must be analysed in an integrated manner. Given the limitations to the number of samples that can be obtained per animal, non-linear mixed effects modelling techniques are recommended for the assessment of concentration–effect relationships (see Box 1). Population PK models can be developed to predict systemic or target drug exposure at the time at which PD measurements are performed. In addition, optimal design concepts can be applied that overcome operational limitations to the sampling scheme, ensuring maximisation of the information gathered in the experiment [60].

In contrast to the linear regression methods applied to most dose–response curves, which are based primarily on observed experimental variables, non-linear mixed effects techniques rely on model parameterisation, which can facilitate the distinction between system- and drug-specific properties. This subtle conceptual difference in data analysis gives a rational basis for ranking compounds and enables pre-clinical findings to be translated to the clinical situation, assuming the same model is applicable across species.

PKPD models

Different approaches are available that account for the description of drug concentrations, drug effects and disease processes [61]. Direct models can be applied when plasma PK can be linked to the PD effects at any given time. Delays between response and PK in plasma because of slow biophase equilibration can be described by effect compartment models [62,63]. Indirect response models can be used to account for the PD of drugs that act by inhibition or stimulation of endogenous mediators [64–66]. More-complex transduction mechanisms can also be modelled by incorporating a so-called transit compartment model [67]. Disease progression models require semi-mechanistic or mechanistic models to enable clear distinction between drug- and system-specific parameters [68,69]. Various mathematical models can be implemented using different parameters for drug effect. Some of these aforementioned models are presented in Box 2. Further details about ongoing efforts in the characterisation of PKPD relationships are also mentioned in Ref. [41]. Interestingly, these concepts have been used more often for the analysis of clinical data (Fig. 5). Application of these concepts to pre-clinical models is scarce in the published literature. Recently, Bender *et al.* have modelled the effects of gabapentin in the CCI model (Fig. 6). Using appropriate protocol design and advanced modelling techniques, the authors demonstrate the predictive performance of a model describing variability in treatment effect across different dose groups for two different endpoints (paw withdrawal threshold and static allodynia).

BOX 1

Non-linear mixed effects modelling. For further details on quantitative pharmacological methods see Refs [80,81]

In non-linear mixed effects modelling, two types of parameters are estimated:

(i) Fixed effects, which are represented by parameters or factors usually explaining the correlation between the dependent and independent variables. These parameters define the structure of the model (e.g. a sigmoidal curve) and their estimates reflect the typical value in the overall population.

(ii) Random effects, which constitute the stochastic component of the mixed effects model. Random effect distributions can be identified for fixed effects with the objective of describing inter-individual and possibly inter-occasion variability. In addition, the residual error represents all the variability that cannot be described by the inter-individual and inter-occasion terms (e.g. measurement error).

Scaling and dose rationale in humans

As indicated previously, one of the main objectives of using a model-based approach is to identify parameters that can be used subsequently to translate response from animals to humans. A direct application of the concept is the prediction of the efficacious exposure range and scaling of the dose across species. To this

BOX 2

Pharmacodynamic models [81]

Direct linear model

$$f(baseline_{ij}, slope_{ij}) = baseline_{ij} + slope_{ij} \times X_{ijk}$$

where $baseline_{ij}$ and $slope_{ij}$ are the parameters to be estimated and and X_{ijk} is usually a measure of drug exposure (e.g. plasma concentration, AUC) corresponding to the observed pharmacological effect y_{ijk}.

Direct E_{max} model

$$f(baseline_{ij}, E_{max\,ij}, EC_{50\,ij}) = baseline_{ij} + \frac{E_{max\,ij} * X_{ijk}}{EC_{50ij} + X_{ijk}}$$

where $baseline_{ij}$, $E_{max\ ij}$ and $EC_{50\ ij}$ are the parameters to be estimated and X_{ijk} is usually a measure of drug exposure (e.g. plasma concentration, AUC) corresponding to the observed pharmacological effect y_{ijk}.

Sigmoid E_{max} model

$$f(baseline_{ij}, E_{max\,ij}, EC_{50\,ij}) = baseline_{ij} + \frac{E_{max\,ij} * X_{ijk}^{\gamma_i}}{EC_{50\,ij}^{\gamma_i} + X_{ijk}^{\gamma_i}}$$

where $Baseline_{ij}$, E_{maxij}, EC_{50ij} and γ_i are the parameters to be estimated and X_{ijk} is usually a measure of drug exposure (e.g. plasma concentration, AUC) corresponding to the observed pharmacological effect y_{ijk}.

The subscripts *i*, *j* and *k* indicate the individual subject *i*, at the time *j* and occasion or period *k*, respectively.

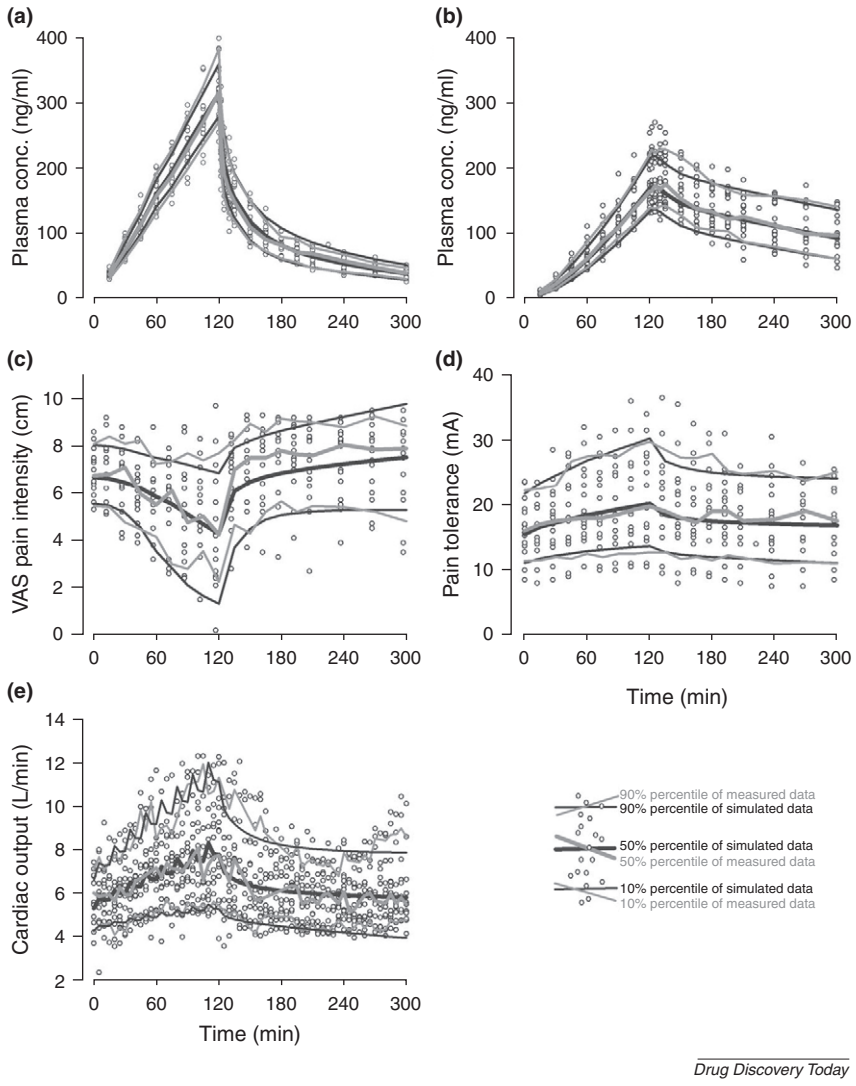

FIGURE 5

Predictive performance and accuracy of a PKPD model describing the effects of S(+)-ketamine in healthy volunteers. Simulations were performed with the S(+)-ketamine parameters obtained from the study data. Despite between-subject variability, it is clear from the panels that the PKPD model describes treatment response. **(a)** S(+)-ketamine concentration; **(b)** S(+)-norketamine concentration; **(c)** heat pain intensity; **(d)** electrical pain tolerance; **(e)** cardiac output. The lines represent the 10th, 50th and 90th percentiles for simulated (black lines) and measured responses (gray lines). The symbols are the actual observations in healthy volunteers. VAS, visual analogue. Reprinted with permission from [75].

purpose, the concentration–effect relationships obtained in animals can be used to estimate the putative clinically effective dose range, taking into account the differences in protein binding and tissue equilibration kinetics across species. Given that differences in endpoints across species are not always correlated with each other in a fully mechanistic manner, derived parameters such as EC_{20}, EC_{80}, EC_{90} should also be considered. This concept has been elegantly illustrated by Huntjens *et al.* in the evaluation of the anti-inflammatory pain by COX-2 inhibitors [13].

Given the complexity of pain signalling and processing, different results are observed depending on which component or step is being quantified. We strongly believe that the answers regarding translational research lie in the use of experimental conditions that warrant construct validity [33,70]. In fact, we defend a paradigm shift in the approach to interspecies scaling based on target and/or receptor occupancy. Receptor theory concepts can be a useful tool for that purpose, enhancing the potential predictive value of a model-based approach [56]. Moreover, the use of target occupancy as a marker of pharmacology can elucidate situations in which a mismatch exists between target activation and effect, as in the case of poor sensitivity of the behavioural endpoint. The cold hyperalgesia model is one such model wherein lowering the temperature below the threshold for nociception does not always provide for a better differentiation between the injured and normal paws of FCA rats [71]. In this context, a mismatch in the degree of target activation across species might explain differences in sensitivity in endpoints as well as in potency. Hence, it would be desirable and useful to integrate receptor binding, which is a measure of receptor activation or inactivation (in the case of an antagonist), with downstream markers of pharmacology and overt behaviour. From an experimental perspective, the use of imaging techniques would support the identification of differences as well

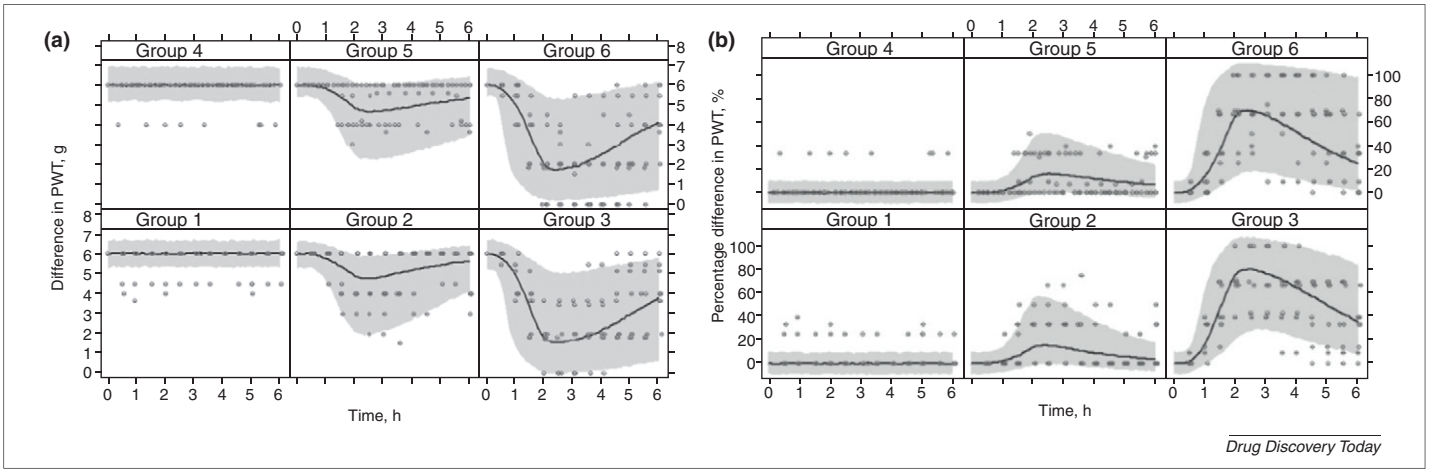

FIGURE 6

Time course of response for individual animals (circles) and the predicted population profiles (black lines) in the CCI model after administration of different doses of pregabalin alone or in combination with sildenafil. The drug effect on statistic allodynia was characterised by (a) the difference in PWT (i.e., between the injured and uninjured paw) and (b) the percentage difference in PWT (i.e., the difference between the two paws divided by the difference at baseline). In both cases, a continuous pharmacodynamic model was used to describe the concentration–effect relationship of pregabalin. The treatment groups included: (1) saline, (2) pregabalin 4 mg/kg/h, (3) pregabalin 10 mg/kg/h + sildenafil, (4) sildenafil, (5) pregabalin 1.6 mg/kg/h + sildenafil and (6) pregabalin 4 mg/kg/h + sildenafil. Model predictions (black lines) include the 90% prediction interval (shaded areas) [57]. Visual assessment of the random dispersion of the data within the predicted intervals provides evidence of the performance of the model. Reprinted with permission from [82].

as correlations across species. It should be noted that incorporation of the binding kinetics in the evaluation of novel compounds also offers an opportunity for better understanding of secondary pharmacological effects and adverse events. By contrast, one should consider secondary pharmacology and safety findings as a proxy for target engagement when evidence cannot be derived experimentally. This concept is illustrated by the use of *N*-methyl-D-aspartate (NMDA) antagonists (e.g. ketamine) and alpha-2 agonists (e.g. clonidine). Although tolerability in humans will ultimately determine dose selection, drug-induced adverse events are usually dose-dependent and specific markers of pharmacological action.

Challenges and limitations

It is beyond doubt that numerous challenges must be overcome to translate pre-clinical findings into predictors of clinical efficacy. Here, we have introduced the use of PKPD as a tool for bridging the translational gap but this approach itself has limitations. Animal models of pain are often technically laborious, making inter-individual variability an intrinsic feature of these models [72]. Drug concentrations at the biophase are not constant over the course of treatment and can be altered by changes in the underlying pathophysiology or natural evolution of the experimental injury [73]. These variations can lead to biased estimates of parameters such as EC_{50} or E_{max}. Some aspects of disease are difficult to model; for instance NP symptoms wax and wane over time with intervals ranging from one day to as long as two or three months [74]. There are also feasibility limitations in terms of the number of samples that can be collected and of the overall duration of follow-up.

All these hurdles raise the question about whether R&D requires more-extensive use of surrogate human models to ensure accurate prediction of clinical efficacy, forsaking pre-clinical experiments. Human models can reproduce many of the symptoms and sensory features in patients of NP [75]. By design, however, these models lack construct validity, inducing acute plasticity (i.e. phase 2 pain instead of long-lasting and irreversible modification of the nociceptive pathway), which is a hallmark of NP [76]. In other words, current human models mimic sensory symptoms reflective of NP and nociceptive pain, yielding results that are relevant but not specific to NP [25].

Discussion and conclusions

Empiricism has dominated target identification and screening of compounds for NP. Moreover, the existing models of behavioural pain response have been developed under the assumption that face validity suffices to translate drug effects from animals to humans, ignoring the requirements for accurate characterisation of PKPD relationships [77]. Four main factors can be identified that explain the high attrition rate in this therapeutic area. First, the lack of sufficient understanding about the mechanisms of disease in humans prevents the identification of suitable markers of pharmacological activity. Such (bio)markers could be used in lieu of behavioural measures, which often misrepresent clinical symptoms in NP patients. Second, summaries of treatment effect are limited to qualitative estimates of drug action, most of which cannot be translated directly to humans. Third, experiments continue to assess dose–response curves, ignoring differences in PK across species. Fourth, the duration of the effect and its relationship to target or biomarker engagement is completely set aside.

Our review shows that every claim regarding the translational value of a method requires comprehensive evaluation of the underlying PKPD relationship. Only at this point it is possible to discern whether the lack of predictive value can be assigned to intrinsic limitations in experimental models (e.g. construct validity or poor sensitivity of the behavioural endpoints). We have also highlighted the relevance of the differences in the development of behavioural pain symptoms caused by injury or noxious stimuli in these models, as compared to the onset and progression of symptoms observed in humans. Although it takes a few weeks for the

disease to be induced in an animal model, in patients this can take years to manifest [78]. A possible explanation for these discrepancies includes the differences in signalling mechanisms. Thus, one should accept that animal models might never be able to mimic all aspects of disease in humans and could be limited as a tool for qualitative distinction between active and inactive compounds.

In summary, the use of non-linear mixed-effects modelling to characterise PKPD relationships in early drug development enables a less empirical rationale for the selection of doses to be tested during first-in-human and proof-of-concept (POC)/Phase II studies. Model-derived parameters can be used to estimate the effective dose range in humans assuming that the free concentration of the drug at the biophase can be compared across species. Our examples also illustrate that the translational value of a model-based approach relies on the choice of appropriate parameters. An integrated approach that takes into account target engagement and markers of pharmacology as the basis for the dose rationale in clinical trials is needed without further ado.

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