Accepted: 6 June 2017

DOI: 10.1111/exd.13392

ORIGINAL ARTICLE

WILEY Experimental Dermatology

Characterization of the chloroquine-induced mouse model of pruritus using an automated behavioural system

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Abstract

Pruritus is a major symptom of several dermatological diseases but has limited therapeutic options available. Animal models replicating the pathophysiology of pruritus are needed to support the development of new drugs. Induction of pruritus by chloroquine (CQ) in mice is widely used, although, as with similar models, it has low throughput and does not distinguish between antipruritic effects and confounding factors such as sedation. To overcome these issues, we incorporated into the model an automated system that measures both scratching and locomotor behaviour simultaneously. We combined this system with the determination of CQ levels in different tissues to understand the impact of the route of CQ administration on the pruritogenic response. We concluded that whereas oral CQ does not induce pruritus due to insufficient skin levels, the bell-shaped curve of pruritus observed following subcutaneous administration is due to toxicity at high doses. We validated the model with several drugs currently used in humans: nalfurafine, aprepitant, cyproheptadine and amitriptyline. By comparing the effects of the drugs on both scratching and locomotor activity, we concluded that nalfurafine and aprepitant can exhibit efficacy at doses devoid of central effects, whereas central effects drove the efficacy of the other two drugs. This was further confirmed using non-brain-penetrant drugs. Moreover, as anticipated, anti-inflammatory drugs showed no efficacy. In conclusion, the use of an automated integrated behavioural assessment in CQ-induced pruritus makes the assay suitable for screening purposes and allows for a correct interpretation of the antipruritic effect of the compounds evaluated.

KEYWORDS
animal model, drugs, locomotion, scratching, screening

1 | INTRODUCTION

Pruritus, or itch, is defined as an unpleasant sensation that elicits the desire to scratch.[1] It is the most frequent symptom in dermatology and can be caused by a variety of clinical conditions such as infections or metabolic disorders.[2] Pruritus lasting beyond 6 weeks is defined as chronic pruritus[3,4] to differentiate it from the acute form. The overall prevalence of chronic pruritus is estimated to be approximately 13.5%, although it can reach up to 50% in dermatological diseases and can also be above 25% in chronic kidney or hepatobiliary diseases.[5] Chronic pruritus represents a worldwide burden in different patient populations of all ages; it is a debilitating symptom and has a dramatic impact on patients' quality of life.[6]

Antihistamines are the most widely used systemic antipruritic drugs, although they have been proven to have limited efficacy in chronic pruritus.[7] Drugs that target the opioid receptor system, such as the mu antagonists (naltrexone, naloxone) and the kappa agonists (nalfurafine),[7,8] are also used in certain pathologies. The lack of a

therapeutic armamentarium against pruritus has prompted the off-label use of drugs used in neuropathic pain (such as gabapentin and pregabalin),[9] depression (amitriptyline, paroxetine, mirtazapine and doxepin) or emesis (aprepitant).[8] In many cases, the drugs used have modest efficacy or significant side effects. Both circumstances emphasize the need for more specific therapies.

Basic research is essential to understand the mechanisms underlying chronic pruritus. Translational models of non-histaminergic itch are required for the development of novel drugs. Chloroquine (CQ) is an antimalarial drug widely used in African patients. CQ induces generalized pruritus as most common side effect and is a cause of treatment discontinuation.[10,11] In mice, injection of CQ into the nape of the neck also induces strong itch behaviour.[12] CQ has been described to act by binding to the Mas-related G protein receptor (Mrgpr), mainly expressed in sensory neurons. MrgprA3 is the CQ receptor in mice, and MrgprX1 is the human orthologue.[13] The CQ model has been used to study the pathophysiology of histamine-independent pruritus.[13,14] However, to date, its use has been limited and no extensive validation with pharmacological tools is available. One potential explanation is that scratching, used as a surrogate endpoint of itch severity, is generally quantified manually by analysis of video recordings of animals.[15] Such a procedure is tedious, time-consuming and has very low throughput.

Our aim has been to further characterize the mouse CQ model and explore its utility for the screening of drugs using an automated recording system that integrates pruritus and locomotion behaviours (Laboras, Metris). The model herein described can be considered a relevant preclinical assay that could help accelerate the development of new therapies.

2 | METHODS

2.1 | Compounds

Chloroquine phosphate, amitriptyline, cyproheptadine, dexamethasone and capsaicin were purchased from Sigma-Aldrich (Ref. PHR1258, A8404, C6022, D1756 and M-2028, respectively). Aprepitant was purchased from CMS Chemicals Limited (Ref. FA17961). Nalfurafine, desloratadine, asimadoline and tofacitinib were synthesized at the Medicinal Chemistry Department of Almirall R&D (Sant Feliu de Llobregat, Spain).

2.2 | Animals

Adult male C57BL/6JRj mice (body weight 20-25 g) were purchased from a commercial breeder (Janvier Labs, France) and housed at the animal facilities of Almirall throughout the study. The mice were housed in ventilated cages with an inner floor area of 500 cm^2 and kept in a room with controlled conditions of light, ventilation, temperature (22±2°C) and humidity (55±10%). A standard rodent diet and water were available ad libitum throughout the study. The care of animals was undertaken in compliance with the European Committee Directive 2010/63/EU and the Spanish and autonomous Catalan laws. All procedures were performed according to the ARRIVE guidelines, with the approval of the Animal Experimentation Ethical Committee of Almirall, and in compliance with the European Committee Directive 2010/63/EU and the Spanish and autonomous Catalan laws.

2.3 | Chloroquine-induced pruritus model in mice

Pruritus was induced in C57BL/6 male mice by a subcutaneous (s.c.) injection of 50 μL of CQ at different concentrations in the nape of the neck. No anaesthesia was used for the injection to avoid interference in the assay. The doses tested were 8, 16 and 32 mg/kg. CQ was carefully dissolved in 0.9% saline solution for all experiments. In another set of experiments, CQ was administered orally at the same doses. Prior to CQ injection, the mice were placed in cages for acclimation for 30 minutes, and immediately after CQ s.c. injection, they were individually transferred to behavioural cages. As a control, capsaicin was dissolved at 0.2 mg/mL in 0.4% ethanol in saline, and 50 μL was injected s.c. in the nape, as above. The number of scratching bouts and distance run for each animal were registered over a span of 30 minutes after CQ challenge and were analysed. The number of animals used for each experimental condition was between 6 and 12.

2.4 | Experimental conditions

During the experiments, mice were habituated in normal home cages (type 2, polycarbonate) on top of an antivibration shelf integrated into a closed Faraday cage, to reduce potential interference in behavioural measures by electrical noise. Behavioural cages were placed on the sensor platform of the LABORAS system (Laboras, Metris Hoofddorp).[16] The cages on top of the measurement platform were placed and secured in fixed positions. Normal bedding material was added to the cages to increase animal welfare and reduce stress. Only one animal was monitored in each cage at a time.

2.5 | Behavioural studies

Animals were removed from the study cage for subcutaneous injection of CQ and returned to the same cage for videotaping with an EthoVision system (Noldus Information Technology) and/or for automatic registry with the LABORAS system at the same time. The video cameras were placed on top of the cages. Scratching bouts were counted manually from a video recording. A scratching bout was defined as repetitive fast movement of the hindlimb of the mouse, rubbing the neck or area of injection. Two independent well-trained observers analysed videos corresponding to 24 different animals for the head-to-head comparison.

LABORAS technology is a non-invasive technology based on vibration and force signal analysis to determine both the behaviour and the position of the animal over the course of the experiment.[16] Signal analysis and pattern recognition of scratching bouts are based on the frequency, which is higher than for most other behaviours and occurs in the range of 14-26 Hz; the variation of the signal peak value, strong and relatively constant for each paw movement; and the shape of the signal. Scratching can be distinguished by the LABORAS software from other behaviours such as grooming (animal shakes, wipes or licks

its fur, snout, ears, tail or genitals with forelimbs or head) or rearing (animal stands upright on its hind legs) that have lower frequencies and less repetitive patterns. While scratching typically occurs at the same animal position, displacement of the animal can be followed and converted to an XY position track from the signals of the two sensors.

2.6 | Determination of CQ levels in mice

Mice were treated with CQ by the s.c. (16 and 32 mg/kg) or oral route (32 mg/kg), and plasma, brain and skin biopsies were collected at the end of the experiments. Skin samples were obtained from the site of injection of CQ in animals treated s.c. and from the same zone in animals administered orally. Plasma samples were deproteinized with acetonitrile/0.2% TFA. Brains were homogenized with methanol (1:4, w/v) and sonicated. Skin biopsies were extracted with acetonitrile/0.2% TFA using a FastPrep (MP Biomedicals, Santa Ana, CA, USA). Following centrifugation of the samples, the supernatants were analysed by UPLC-MS/MS using a Waters Xevo (Milford, MA, USA).

2.7 | Effects of several treatments on pruritus and locomotor activity

Compounds or vehicle controls (0.5% methylcellulose+0.1% TWEEN 80 in water) were administered to mice by the intraperitoneal (i.p.) or oral (p.o.) route at different doses. The time for optimal dosage was defined for each drug according to its pharmacokinetic profile to achieve maximal exposure during the experiments. Prior to CQ administration, the mice were placed in cages for acclimation for 30 minutes. Pruritus was induced by s.c. injection of 50 μL of CQ at a dose of 16 mg/kg, and the mice were immediately allocated individually to behavioural cages, as described. The number of scratching bouts and distance run for each animal were recorded during 30 minutes after CQ challenge. Nalfurafine, cyproheptadine, desloratadine, asimadoline, tofacitinib and dexamethasone were administered by oral gavage. Amitriptyline and aprepitant were administered intraperitoneally. The number of animals used for each experimental condition was between 6 and 12.

2.8 | Statistical analysis

Statistical significance was analysed using repeated-measures comparisons, using one-way or two-way analysis of variance and a post hoc Dunnett's or Tukey's correction; $P<.05$ was considered significant. These analyses were performed using GraphPad Prism software (La Jolla, CA, USA).

3 | RESULTS

3.1 | Comparison between manual and automatic procedures

A head-to-head comparison of the manual (videotaped) and automatic scratch counts was performed from the same 24 animals to validate the system (data not shown). The two measurements showed a good correlation, with Spearman's r equal to 0.61 (n=24, $P<.005$, F test). The automated platform counted an average of threefold fewer scratches compared to manual counts (manual values ranged from 124 to 451, whereas automatic values ranged from 45 to 186). This could be explained since the automated system integrates the signals over periods of time, and one scratching behaviour could be defined over a period in which two or three repetitive scratching movements would be counted by the observer.

3.2 | Dose-response effect of CQ in pruritus and locomotor activity

CQ at doses ranging from 8 to 32 mg/kg induced an increased scratching response after s.c. administration (Figure 1A). At the highest dose tested, however, the number of scratching bouts was not significantly different compared with the vehicle group, reproducing the bell-shaped scratching response described for C57BL/6 mice,[12] further validating the automatic count. CQ caused a significant effect in locomotor activity only at the 32 mg/kg dose (Figure 1B), indicating that this s.c. dose could be toxic to mice. When CQ was administered orally, no effect on either scratching or locomotion was observed (Figure 1C, D). Pruritus was also not observed up to 5 hours after oral CQ administration (data not shown) nor after higher doses up to 100 mg/kg, in agreement with previous reports.[17]

To assess the specificity of the scratching response, we injected capsaicin into the nape of mice. At doses similar to reported ones,[18] capsaicin did not induce scratching in our model (data not shown).

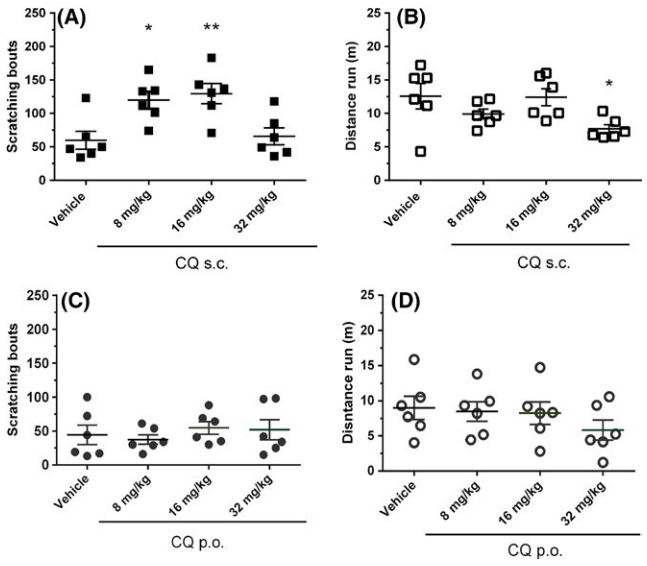

FIGURE 1 Scratching and locomotor activity measurements after CQ administration in mice. Animals were treated with increasing concentrations of CQ (8, 16 and 32 mg/kg) either s.c. (A and B) or p.o. (C and D) and placed in the behavioural cages for 30 min. Cumulative scratching bouts and cumulative distance run by each animal in 30 min are plotted against CQ dose. Mean±SEM is shown. Statistical evaluation was performed with one-way ANOVA followed by Dunnett's t test; *$P<.05$, **$P<.005$. These results are representative of a set of two independent experiments using six animals per group

Capsaicin injected into the cheek of mice was reported to induce wiping but not scratching.[19] We could not assess the wiping response with the LABORAS as it is associated with grooming behaviour and could not be quantified separately.

3.3 | Quantification of CQ levels in mouse tissue samples to understand lack of pruritus in different experimental conditions

As described above, a dose of 16 mg/kg s.c. induced pruritus with no effect on locomotor activity. In contrast, at a dose of 32 mg/kg, CQ did not induce pruritus irrespective of the route of administration. Moreover, a reduction in spontaneous locomotion was observed following 32 mg/kg s.c. administration, whereas the same was not observed following p.o. administration, suggesting that the reason behind the lack of pruritus response could be different in the two groups. We hypothesized that the route of administration could lead to differential exposure to CQ in the different tissues, and such exposure could explain the differential results. We determined the levels of CQ in plasma, brain and skin of three groups of mice: the two 32 mg/kg dose groups and the 16 mg/kg s.c. dose. As shown in Table 1, the CQ levels of the 32 mg/kg p.o. in plasma were significantly higher (2.5-fold) than those of the 16 mg/kg s.c. group, while brain levels were fairly similar. However, levels in the skin were 45 times lower in the p.o. than in this s.c. dose. This difference might explain the lack of efficacy in pruritus.

Skin levels of CQ in the 32 mg/kg s.c. group were twofold higher than those of the pruritogenic 16 mg/kg s.c. dose, and still animals didn't scratch. However, the CQ levels in plasma and brain at 32mg/kg s.c. were higher than in the other two groups having no reduction of locomotion. This suggested that increased brain levels were likely responsible for the effects on locomotion.

3.4 | Pharmacological characterization of the model using the automated system

As shown in Figure 2A, the automatic count reproduces the nalfurafine dose-response reported.[17] Low doses of the kappa opioid receptor agonist (0.03 mg/kg) moderately inhibited scratching (44%) without significant reduction in spontaneous locomotor activity (21%). At the highest dose tested (0.1 mg/kg), the drug induced scratching inhibition up to 83%, while locomotor activity was reduced by 53%, revealing that there is a narrow therapeutic margin for this drug. Cyproheptadine, a first-generation antagonist at H1 and serotonin receptors, dose-dependently inhibited pruritus (97% at the highest dose of 10 mg/kg) to the same extent as locomotor activity (99% at this same dose) (Figure 2B). Amitriptyline, a tricyclic antidepressant, at a dose of 10 mg/kg potently inhibited both scratching and locomotor activity by 89% and 81%, respectively (Figure 2C), indicating that central effects were underlying its efficacy in pruritus. Aprepitant, an NK1 receptor antagonist, showed a significant effect on scratching at 100 mg/kg, the highest dose tested (52%), with no alteration of spontaneous locomotion, indicating a true antipruritic effect (Figure 2D).

To further assess the role of the CNS in the efficacy of kappa opioid agonists and H1 receptor antagonists, two compounds with low brain penetration were tested. Asimadoline, a kappa opioid receptor agonist,[20] and desloratadine, a second generation H1 receptor antagonist,[21] were selected for this purpose. As shown in Figure 3A, asimadoline showed no significant antipruritic effect nor inhibition of locomotion at low doses of 0.1 mg/kg reported to have no sedative effects.[22] Desloratadine showed no effect on pruritus nor on locomotion when tested at low doses of 0.05 mg/kg reported to be efficacious in bronchial hyperresponsiveness models,[23] and not even at doses up to 10-fold higher (Figure 3B). Therefore, our results demonstrate that neither of the low-brain-penetrant compounds showed antipruritic effects at low non-sedative doses.

Anti-inflammatory drugs such as the JAK inhibitor tofacitinib (10 and 30 mg/kg) or the corticosteroid dexamethasone (5 mg/kg) showed minor (20-30%) antipruritic effects in this model (data not shown). These doses were selected because they demonstrated efficacy in mouse models of inflammation.[24]

4 | DISCUSSION

Relevant animal models are needed to support drug development for pruritus. Models need to be suitable for screening purposes,

TABLE 1 Relationship among efficacy, toxicity and levels of CQ in relevant tissues after its s.c. or p.o. administration to mice

CQ (mg/kg)	Route	S/L	Plasma (ng/mL)	Brain (ng/g)	Skin (ng/g)
32	p.o.	No/no	2400±389	1142±259§	322±90§
16	s.c.	Yes/no	1098±96$^{**,\#}$	1717±123**	14 488±2200$^{*,\#}$
32	s.c.	No/yes	3425±363	3788±229	32 050±6950

CQ levels in the three tissues were quantified by UPLC-MS/MS at the end of the pruritus experiments. We compared the levels of CQ in the plasma, brain and skin of three treatment groups that exhibited different behaviours in terms of induction of scratching (S) and inhibition of locomotion (L). Low levels in the skin may explain the lack of pruritus in the group treated p.o. Excessive exposure to CQ in the plasma and/or brain may have produced a toxic effect that prevented animals from scratching when treated at 32 mg/kg s.c. The results are expressed as the mean±SEM from six animals per group. Statistical analysis was performed using one-way ANOVA with Tukey's test to correct for multiple comparisons.

$^{*}P<.05$ and $^{**}P<.0005$ compared with 32 mg/kg s.c., $^{\#}P<.05$ compared with 32 mg/kg p.o. and $^{\S}P<.0005$ for 32 mg/kg p.o. compared with s.c. No significant differences were observed between CQ plasma levels after p.o. and s.c. administration.

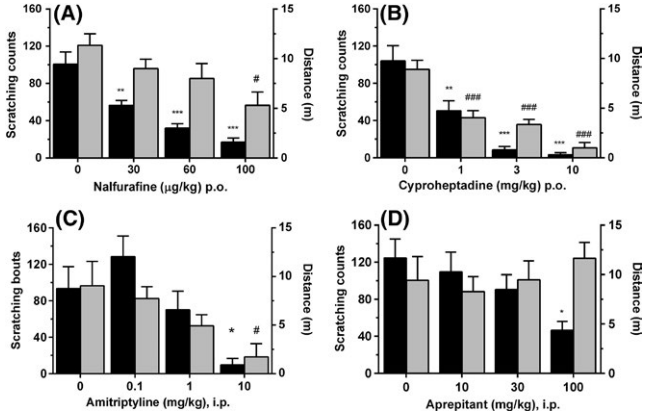

FIGURE 2 Pharmacological validation of the CQ model. Dose-response effect of nalfurafine (A), cyproheptadine (B), amitriptyline (C) and aprepitant (D) on number of scratching bouts (black bars) and locomotor activity (distance run, grey bars). At least 6 animals were used for each treatment group. A, Nalfurafine was administered to mice at 30, 60 or 100 μg/kg by gavage 45 min before s.c. injection of CQ. Significant dose-dependent inhibition of pruritus was observed from the lowest dose. Only a minor effect on locomotion at the highest dose was observed. B, Cyproheptadine was orally administered to mice at 1, 3, or 10 mg/kg 30 min before s.c. injection of CQ. Both pruritus and locomotion decreased with increasing doses. C, Amitriptyline was administered to mice at 0.1, 1, or 10 mg/kg i.p. 30 min before s.c. injection of CQ. Only the highest dose was able to inhibit pruritus, and that dose also inhibited locomotor activity. D, Aprepitant was administered to mice at 10, 30, or 100 mg/kg i.p. 45 min before s.c. injection of CQ. Only the highest dose was able to inhibit pruritus by 50%, and interestingly, no decrease in locomotor activity was detected. One-way ANOVA followed by Dunnett's test was applied for statistical evaluation. Statistically significant differences between drug-treated and vehicle-treated animals are reflected; *P<.05, * or # p<.05, ** p<.01, *** or ### p<.001,***P<.0005

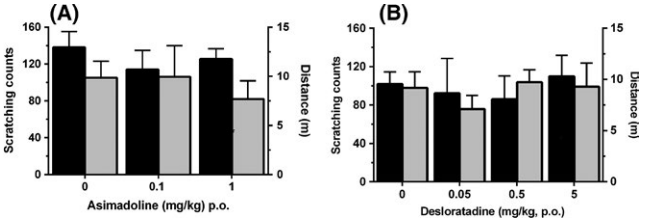

FIGURE 3 Low-brain-penetrant compounds are not efficacious against pruritus. Dose-response effects of asimadoline (A) and desloratadine (B) on number of scratching bouts (black bars) and distance run (grey bars) for 30 min after s.c. injection of CQ. At least 6 animals were used for each treatment group. A, Asimadoline was administered to mice at 0.1, 1 mg/kg p.o. 30 min before s.c. injection of CQ. No scratching inhibition was observed at low, non-brain-penetrant doses. Locomotion was not affected either. B, Desloratadine was administered to mice at 0.5 or 5 mg/kg p.o. 1 h before s.c. injection of CQ. Again, these doses that are reported not to be brain penetrant showed no efficacy at inhibiting pruritus, and no effects on locomotor activity were observed

reproducible and able to provide the maximum amount of information in every assay for ethical reasons. Induction of pruritus by injecting a substance in the nape or the cheek of rodents has been used frequently to study the mechanisms involved in itch.[13,14,17,25] Evaluation of pruritus is often done by video recording of the animals, followed by visualization and counting of scratching bouts. These models are simple and short in terms of execution, but the assessment of pruritus is tedious and time-consuming, and results may vary depending on the observer. Several attempts have been made to automatize the procedure,[15,26] but most of the current scientific publications are still using the manual approach.[27,28] Additionally, measurement of scratching alone does not allow for a correct interpretation of the efficacy results. Drug-treated animals may not scratch because they are sedated or because they experience pain. Some authors conduct behavioural tests in parallel to pruritus tests, although using different sets of animals.[29] Simultaneous measurement of scratching and motor activity has been described in a non-invasive, non-commercial system[30] using a similar detector system and pattern recognition to LABORAS. However, that system seems less optimized in terms of platform configuration and pattern recognition software, and motor activity is quantified in terms of time of immobility instead of distance run. Although videotape recording will ultimately remain the standard method to assess scratching, the simultaneous measurement of scratching bouts and locomotion in an automatized way as described herein may provide a solution to two important drawbacks in drug discovery: throughput and interpretation.

Dose-response studies of CQ by the s.c. route using the automated system replicate the same bell-shape curve in pruritus that has been reported with manual counts,[12] with no effect observed at the highest dose tested, 32 mg/kg. Scratching results support the validity of the automatic count, but moreover, results reveal a significant reduction of locomotor activity that is concomitantly observed at this dose, suggesting the occurrence of a toxic effect that would interfere with the animal's need to scratch. It could also be argued that different compartments are responsible for the diminished locomotor activity (blood or brain) and for the lack of skin pruritus, as demonstrated with CQ quantification in the different tissues. Moreover, MrgprA3, the receptor of CQ,[13] is a G protein-coupled receptor, and as such, it can be internalized following agonist binding. It may occur that CQ receptor is desensitized due to the high doses of the agonist in the area of the injection, thus preventing itch signalling.

As previously reported, no effect on scratching behaviour was induced following oral CQ administration,[17] nor did we observe any effect on locomotion. At the highest oral dose assayed, CQ skin levels were extremely low. The CQ receptor, MrgprA3, is exclusively expressed in sensitive neurons in the skin, and the concentration of CQ needed to activate the receptor is reported to be 27.55 μM.[13] The levels we detected in the skin after the oral dose were approximately 1 μM (322 ng/g), clearly insufficient to induce pruritus. On the other hand, the concentration of CQ in the skin following s.c. administration is above that threshold (45 and 100 μM at 16 and 32 mg/kg s.c., respectively). These results further support the idea that CQ-induced pruritus is mediated by MrgprA3 in the skin of mice. Bearing these results in mind, one would suggest that for a drug to be active in this

model, either it should have high skin exposure to be able to counteract these high local levels of CQ, or it must act on the central nervous system, peripherally or centrally. These considerations may help rationalize the value and the limitations of this experimental model.

Pharmacological validation of the CQ model with drugs used in humans was lacking. We selected a set of drugs reported to be efficacious in human pruritus, mainly of renal or dermatological origin.[4,7] Of the six drugs with different mechanisms assayed, only the kappa opioid agonists had been previously tested by the oral route in this model.[17] NK1 antagonists have also been reported to show efficacy in the CQ model after intrathecal administration.[31] A reduced effect of the H1 receptor antagonist desloratadine administered i.p. has also been reported in the model.[32] However, only their effect on scratching and not their effect on locomotion was assessed. Comparison of the dose-response effect of a drug on scratching vs locomotion allowed us to establish the dose at which a net antipruritic effect was achieved. A net antipruritic dose could be defined as one in which a significant reduction of itch is achieved with no effect on locomotion.

Amitriptyline and cyproheptadine showed no net antipruritic dose, as a parallel decrease in both scratching and locomotion behaviour was observed at all doses assayed. No antipruritic effect of the low-brain-penetrant antihistamine desloratadine was observed, suggesting that the CQ model is independent of histamine and that antihistamines work in the model because they induce sedation. Nalfurafine induced a 50% reduction in pruritus at the lowest dose tested, causing only a modest effect on locomotion, and that dose could thus be established as the net antipruritic dose. These results, together with the lack of efficacy obtained with the low-brain-penetrant kappa agonist asimadoline,[20] would indicate that a peripheral effect is minimally or not responsible for the efficacy of this class of compounds. Aprepitant also showed a net antipruritic effect at the highest dose tested, supporting the therapeutic use of NK1 inhibitors in humans. Finally, the lack of effect on CQ-induced pruritus of the two anti-inflammatories tested, tofacitinib and dexamethasone, confirms that inflammation is not involved in the induction of pruritus by CQ. Notably, oral tofacitinib has been reported to reduce pruritus in patients with psoriasis.[33]

The relevance of a certain degree of pruritus inhibition in the mouse model is difficult to assess in terms of translation to humans. The same may be true for the effects on locomotor activity. Species differences in the potency of the drugs against their targets and differential CNS penetration may increase the uncertainty of this translation. Nevertheless, our model and the proposed analyses provide information on the therapeutic indexes of drugs, allowing selection of those that exhibit higher margins.

Although the CQ model is, *sensu stricto*, a model of acute pruritus, it shares two similarities with human chronic pruritus. First, it responds to drugs that have proven efficacious in chronic pruritus in humans, and second, it does not respond to non-sedating antihistamines. The only compounds we have found to induce full inhibition of pruritus are drugs targeting the CNS. This may be a particularity of the CQ model, but it may also be a limitation of chronic pruritus itself. In humans, a drug interrupting itch circuits in the CNS should theoretically be effective in different types of pruritus, irrespective of the underlying condition. If such a CNS target exists and its inhibition does not cause major side effects, many patients suffering from pruritus stand to benefit from it.

The automated recording system used herein can be applied to other models of pruritus. We have recently used it to evaluate pruritus in a mouse model of skin inflammation following repeated topical applications of oxazolone (Pont et al., Unpublished Material) which would allow the screening of topical drugs targeting dermatological pruritus.

Pruritus is a challenging area in terms of drug development. The evaluation of novel drugs in humans relies on subjective measurements (VAS scale).[34] Attempts are ongoing to automatize and quantify measurements through wrist instruments, but are not yet implemented. Preclinical studies such as the one reported here could guide the design of combined measurements. Overall, this approach can help diminish the gap between clear unmet needs in several diseases and drug development in the pruritus arena.

ACKNOWLEDGEMENTS

The authors would like to acknowledge L. Boix, J.L. Gómez and M. de Luca for their excellent technical assistance.

CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTION

GT, CC, PE, BP, NG and AG take responsibility for the integrity of the data and the accuracy of the analysis. GT, AG and NG performed the study concept and design. GT, PE, AG and NG performed the analysis and interpretation of the data. CC and BP involved in the technical support. PE,AG and GT performed the study supervision. GT, AG and NG drafted the manuscript. NG involved in the critical revision of the manuscript.

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How to cite this article: Tarrasón G, Carcasona C, Eichhorn P, Pérez B, Gavaldà A, Godessart N. Characterization of the chloroquine-induced mouse model of pruritus using an automated behavioural system. *Exp Dermatol*. 2017;00:1–7. https://doi.org/10.1111/exd.13392