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

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Introduction

Pruritus is the most common symptom of many dermatological indications and has a large unmet medical need. Current treatments are based on first-generation anti-histamines and off-label use of analgesic and antidepressant drugs, which have limited efficacy.

Itch induced by the antimalarial drug chloroquine (CQ) has been used in mice as a translational model of pruritus..

In pruritus models, scratching is usually evaluated from video-recording and manual counting. This method has two limitations; it is time-consuming and it cannot distinguish pruritus inhibition due to an antipruritic effect or to a CNS effect.

Our aim has been to further characterize the CQ model and explore its utility for the screening of drugs using an automated recording system (Laboras, Metris).

Materials & Methods

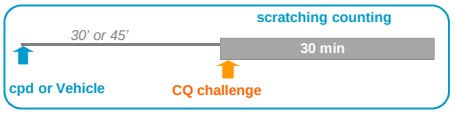
Choroquine induced pruritus model in mice

Pruritus was induced by subcutaneous (s.c.) injection of chloroquine at 8, 16, 32mg/kg (corresponding to 200, 400 and 800µg/mice) or oral route at 100mg/kg to adult male C57BL/6.

To evaluate the antipruritic efficacy of the compounds, pruritus was induced by subcutaneous injection of chloroquine at 16mg/kg.

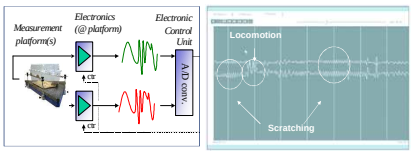
Compounds or vehicle (0.5% methylcellulose + 0.1% Tween 80 in water) were administered by intraperitoneal (ip) or oral (po) route 30 or 45 minutes before chloroquine challenge.

The scratching bouts were counted during 30 minutes after chloroquine challenge. Manual and/or automated recording system (Laboras, Metris) were used to measure scratching and spontaneous locomotion activity.



Scratching and Locomotor activities quantification

LABORAS is a validated and non-invasive technology based on vibration and force signal analysis to determine both the behaviour and the position of the animal along the experiments. Each behaviour has its own unique signature of signal characteristics which can be detected by the software to identify a behaviour, e.g. scratching and locomotion.



Quantification of CQ tissue levels

CQ plasma levels were determined by UPLC-MS/MS after protein precipitation. Brains were homogenized with methanol (1:4, w/v), sonicated and the supernatants analysed by UPLC-MS/MS. Skin samples were extracted with acetonitrile/0.2%TFA using a FastPrep. analysis on Waters Xevo.

References

- Inan, et al. 2004 *Kappa agonists suppress Chloroquine-induced scratching in mice* _ Eur J Pharm 502; 233-237
- Liu et al., 2009 Sensory neuron-specific GPCR Mrgprs are itch receptors mediating choroquine-induced pruritus_ Cell 139; 1353-1365
- Wilson et al 2011 *TRPA1 is required for histamine-independent, Mas-related G protein–coupled receptor–mediated itch* _ Nature Neuroscience 14; 595-603

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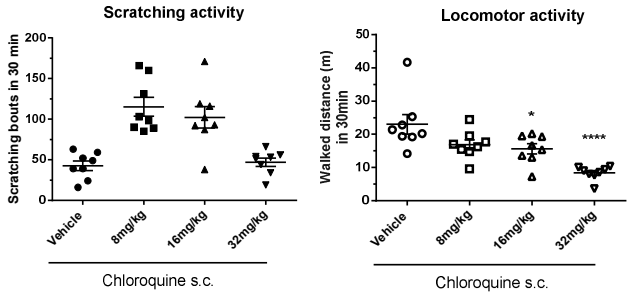
Chloroquine dose-response effect on pruritus model

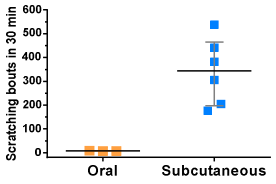


Results expressed as Mean with SEM (n=8)

- Increasing doses of CQ induced a bell-shaped scratching response.
- High doses of CQ induced a significant inhibition of locomotor activity.
- The lack of antipruritic effect at the highest dose of CQ is likely due to a CNS effect.

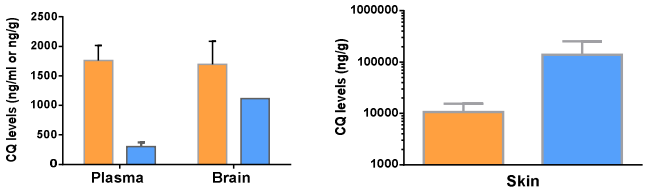
Characterization of the target compartment of CQ

Effect of route of administration



- CQ induces scratching after s.c. but not after oral administration.

Quantification of CQ tissue levels after p.o. and s.c. administration



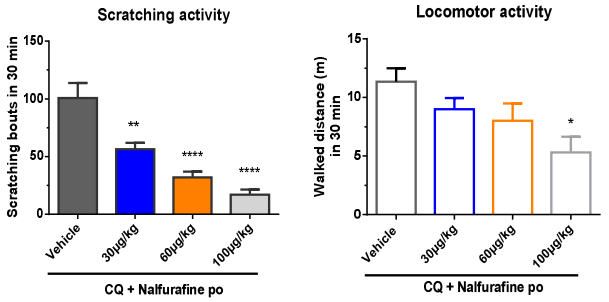
CQ levels in brain and plasma are similar or higher after p.o. than after s.c. administration..However, CQ levels in the skin are much higher after s.c. dosing

p.o
s.c.

- Pruritus induced by CQ is due to a local effect, likely mediated by Mrgpr receptors expressed in the skin.

Pharmacological validation of the model

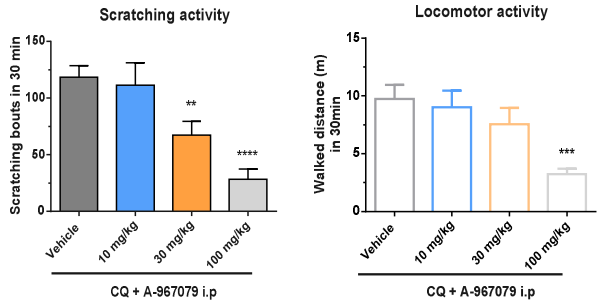
Effect of κ-agonist Nalfurafine on CQ induced pruritus



Results reflect mean with SEM group values (n=6-18)

- As reported, oral Nalfurafine induces a dose dependent inhibition of scratching.
- Only low doses of Nalfurafine inhibit scratching with minor reduction of spontaneous locomotor activity.
- The combined recording of scratching and locomotor activity reveals the potential therapeutic margin of the drug.

Effect of TRPA1 antagonist A-967079 on CQ induced pruritus



Results reflect mean with SEM group values (n=6-18)

- TRPA1 antagonist A-967079 induces a dose-dependent inhibition of scratching.
- Low doses of the compound induced a non significant inhibition of the spontaneous locomotor activity, again revealing the potential therapeutic margin of the drug.
- Pharmacological inhibition of TRPA1 channels confirm results previously reported with TRPA1 deficient mice.

Conclusion

The combined recording of scratching and locomotor activity in an automated system not only increases the throughput of the model but also allows to discriminate between efficacy and CNS effects of the tested drugs.

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