Analysis of diurnal changes in scratch number and frequency (Hz) during a chronic oxazolone-induced inflammation in BALB/c mice

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INTRODUCTION

- The chronic oxazolone-induced dermal inflammation in the mouse is commonly used to test for anti-inflammatory and anti-pruritic compounds. Analysis of oxazolone-induced scratching is often limited to the manual counting of scratching over the first 30 to 120 min after exposure to oxazolone, partly due to the lack of a reliable apparatus for quantifying scratching automatically and partially due to the lack of any data which would justify measuring scratching for longer periods.

-Mouse activity follows a circadian rhythm in which mice sleep during the day and are most active, general movement, grooming, eating, etc, during the night. For this reason the lighting in mouse facilities has a 12 hr light/dark cycle.
- Spontaneous scratching by chronic proliferative dermatitis (CPD) and NC/Tnd mice and picryl chloride-induced scratching by NC mice has been shown to be biphasic with maximum scratching occurring during the "dark" period (1,2,3).

- The questions addressed in this poster are:

a) Does oxazolone-induced scratching in BALB/c mice follow a biphasic rhythm, as reported for spontaneous mouse scratching and picryl chloride-treated mice?

b) What are the effects of a standard anti-inflammatory drug (betamethasone dipropionate) on the scratch pattern during acute and chronic inflammations?

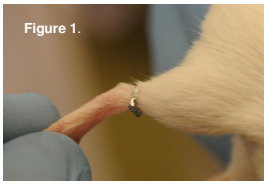
METHODS

Oxazolone treatment; Female BALB/c mice were sensitized to oxazolone by treating the right ears with 1% oxazolone in acetone on day 0. On day 7 mice were challenged with 1% oxazolone in acetone and then, with variations in the exact protocol, every 2 or 3 days up to a maximum of day 18.

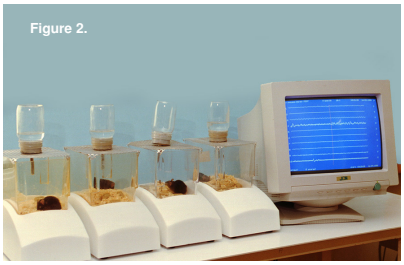
The effect of betamethasone dipropionate; The right ears were exposed 1 x daily to either acetone or betamethasone dipropionate (0.05% in acetone), treatment regime depending on the protocol used.

Measurement of ear thickness; Ear thickness was measured using a Mitutoyo Digimatic Micrometer set at a pressure limit of 2N.

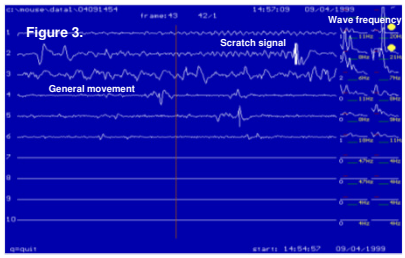
Measurement of scratching and general activity; General activity, the number of scratch events (SEs) and scratch frequency (Hz) were measured for 22 – 24 hours using an automated system that allows mice to remain in a standard mouse cage with bedding, pellets and water (1). See figures 1-3.



A metal ring is placed around the mouse's right hind leg and checked to see that it is not too tight.



Mice are housed in cages containing bedding and pellets and with access to water. The cages are placed on the scratch detection units. Movement of the metal ring generates a signal which is captured by the computer.



The computer analyses the signal each 1.5 sec. Scratch movements are defined as a regular wave with a frequency above 15Hz. General movements are defined as irregular signals with an amplitude of at least 4x the background noise (energy Is expressed in mV).
Only 1 SE or general movement is recorded per 1.5 seconds. The 15 Hz cut-off for detecting SEs is used because;
a) most scratching occurs above this frequency,
b) measurements become less reliable at lower frequencies.

The number of SEs was quantified during the first 22 hours period after exposure to oxazolone in order to standardize the measurement period.

REFERENCES

1. Elliott G, et al. 2000, An automated method for registering and quantifying scratching activity in mice: Use for drug evaluation. J Pharmacol Toxicol Methods. 44: 453-9.
2. Keitaro Ohmori et al. 2011. Circadian Regulation of Scratching Behavior in NC/Tnd Mice, a Mouse Model for Human Atopic Dermatitis. XXII World Allergy Congress, Cancun, Mexico. 4-8 December 2011.
3. Yasushi Hirasawa et al. 2003, Analysis of scratching behavior in a picryl chloride induced atopic dermatitis model in mice. Jpn. J. Allergol., 52:1014-21.

RESULTS

A comparison of general activity (figure 4a), scratching (figure 4b) and ear thickness in control and oxazolone treated mice; the effect of betamethasone dipropionate.

Mice were sensitized to 1% oxazolone on day 0 and challenged with 1% oxazolone on days 7.9.11.16.and 18. On days 7 to 18 mice were treated with either a 0.05% solution of betamethasone dipropionate in acetone or acetone. *The oxazolone control mice were treated with acetone in place of oxazolone and/or betamethasone dipropionate.*

Figure 4a = general activity.

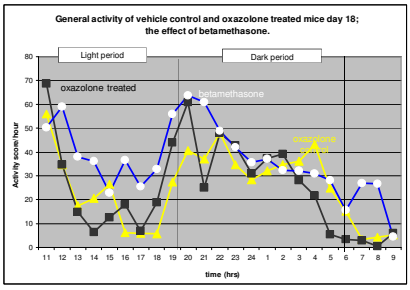
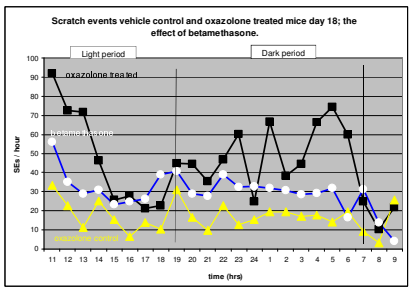


Figure 4b = scratching.

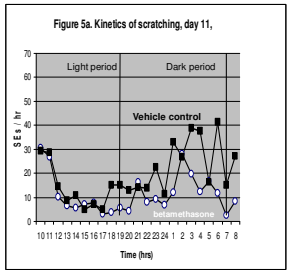


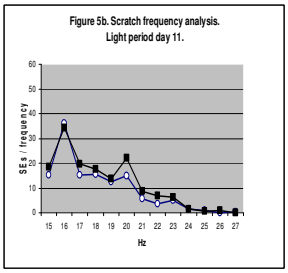
General activity. There was an initial peak of activity following administration of oxazolone which decreased towards 18.00 hrs. There was then a second period of activity during the dark phase. This was observed with all groups.

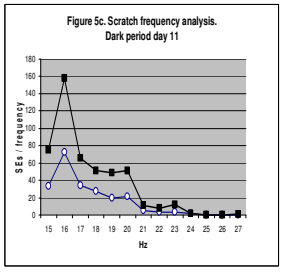
Scratching. Scratching by oxazolone control mice was fairly constant during the whole measurement period. Scratching by oxazolone vehicle and oxazolone betamethasone dipropionate treated mice showed an initial acute peak of activity during the light period following administration of oxazolone, which decreased towards 18.00 hrs. There was then a chronic second period of activity during the dark phase. Betamethasone dipropionate significantly reduced the total number of scratches.

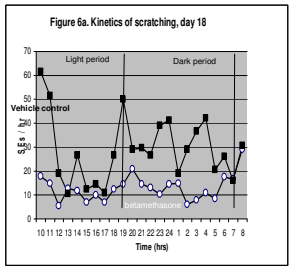
Ear thickness:- oxazolone control = 0.18 mm; oxazolone treated[$] = 0.9 mm; betamethasone dipropionate[*] = 0.53 mm. $ p<0.05 vs oxazolone control and betamethasone groups.

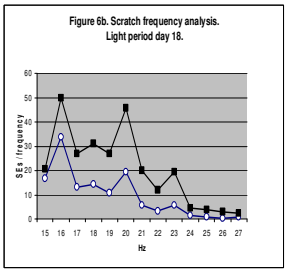
A comparison of scratching parameters and ear thickness during the progress of an inflammation.

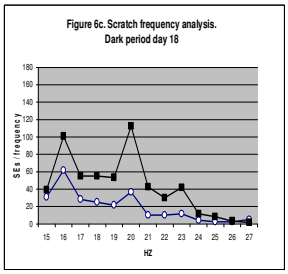


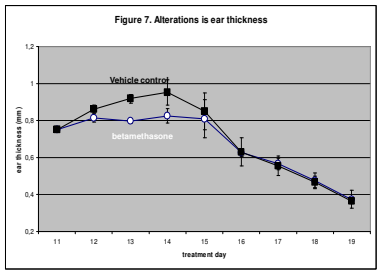












Figures 5 and 6. Mice were sensitized to 1% oxazolone on day 0 and challenged with 1% oxazolone on days 7, 9 and 11. On days 11 to 18 mice were treated with either a 0.05% solution of betamethasone dipropionate in acetone or acetone.

Figure 5. Day 11 scratch analysis. *5a Scratch kinetics, 5b Scratch frequency light period, 5c Scratch analysis dark period.*

Figure 6. Day 18 scratch analysis. *6a Scratch kinetics, 6b Scratch frequency light period, 6c Scratch analysis dark period.*

Figure 7. Alterations in ear thickness

CONCLUSIONS

1. **Neither oxazolone nor betamethasone dipropionate modify general behaviour.**
2. **Oxazolone-induced scratching is biphasic with an early acute "light" phase after application of oxazolone and a later, dark, phase. Scratch stimulation during the "dark" phase, but not the light phase is still present after 48 hrs, data not shown).**
3. **Scratching by vehicle treated mice occurred predominantly up to 20 Hz early in a chronic inflammation (day 11) with a clear stimulation of scratching at 16 Hz during the light and dark periods (Figures 5b and 5c). Betamethasone dipropionate reduced scratching at all frequencies similarly.**
4. **Later in the inflammation, day 18, there was a clear shift in scratching to higher frequencies, between 21 and 25 Hz, during both the light and dark periods. Betamethasone dipropionate preferentially inhibited high frequency scratching.**
5. **Ear thickness is not directly related to scratching.**