Stimulus-evoked and spontaneous pain in Complete Freund's adjuvant-induced arthritis in the rat

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Introduction

Intra-articular injection of complete Freund's adjuvant (**CFA**) in rodents is a well characterized model of monoarthritis and is routinely used for the screening of compounds targeting inflammatory pain. The injection of CFA usually results in an increased sensitivity to noxious stimuli such as heat or mechanical pressure. However, these traditional assays based on evoked readouts may be insufficient to fully encompass the range of clinical pain and do not test the nature of spontaneous pain. Unlike evoked pain response paradigms, the weight-bearing distribution (pedobarograhy) assesses the spontaneous pain as well as pain-related guarding behavior of the inflamed hindpaw.

Objectives

To establish the time course of weight bearing deficits as an objective non-evoked readout for pain in the **CFA model of monoarthritis**.
To comparatively assess the sensitivity of evoked and non-evoked pain responses with their regards to the reversal by analgesic therapy in the CFA model of monoarthritis.

Material & Methods

Female Lewis rats of ~250 g were used and they received 150 µl of CFA into the right tibiotarsal joint. Sham animals received equivalent volume of 0.9 % NaCl. Rats were monitored at different timepoints after the injection of CFA. (days 0, 2, 4, 7, 10, 14, 21 and 28 post-CFA).

Measure of paw swelling

Paw swelling was measured by water displacement plethysmography in anaesthetized rats (3% isoflurane in air). The hindpaw was immersed in a beaker containing water up to the boundary between hairy and nonhairy skin, and the volume displacement was measured by the change in grams of the whole preparation.

Measure of evoked pain

Mechanically evoked pain response of rats (hyperalgesia) was measured using the Randall-Sellito analgesimeter in the non-injected paw (contralateral side) and then in the CFA-injected paw (ipsilateral side). The mechanical nociceptive threshold was defined as the force in grams at which the rat withdraws its paw. The cut off pressure was set to 250 g.

Measure of spontaneous pain

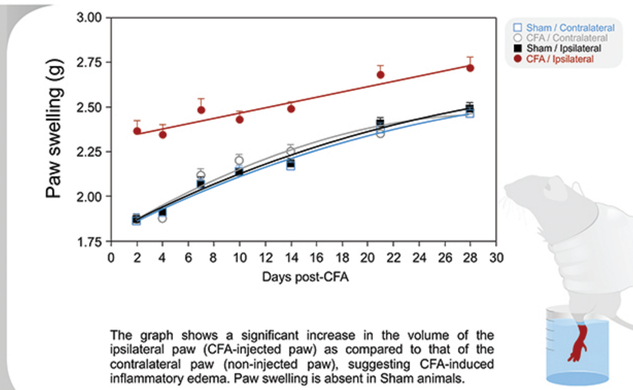
Spontaneous pain was evaluated by monitoring the change in the weight distribution on the hindpaws in freely moving animals over a 5 minutes period within the recording box.
Unilateral gait deficit expressed as a ratio between the weight recorded in the ipsi- and the contralateral hind paw was considered as an indicator of the intensity of spontaneous pain.

Analgesic treatment

Morphine (3 mg/kg) was used as analgesic treatment. The effect of morphine treatment was studied at day 4 post-CFA. It was administered subcutaneously 45 minutes before the measure of pain in CFA-injected rats.

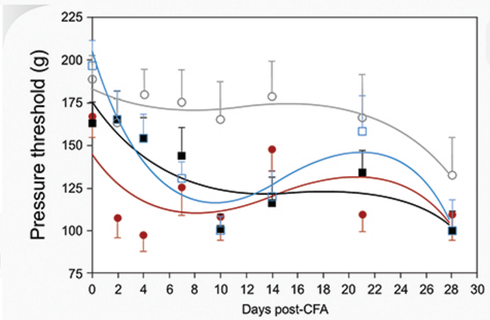
Results

Figure 1: CFA-induced paw swelling



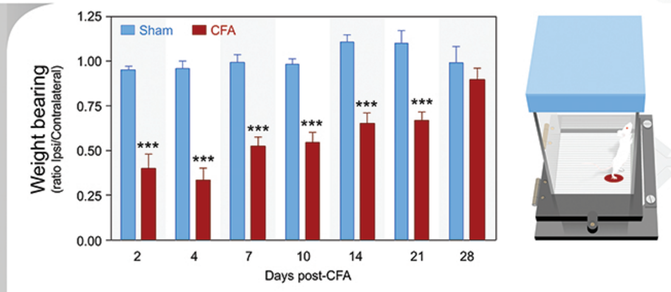
The graph shows a significant increase in the volume of the ipsilateral paw (CFA-injected paw) as compared to that of the contralateral paw (non-injected paw), suggesting CFA-induced inflammatory edema. Paw swelling is absent in Sham animals.

Figure 2: Evoked pain in response to mechanical hyperalgesia



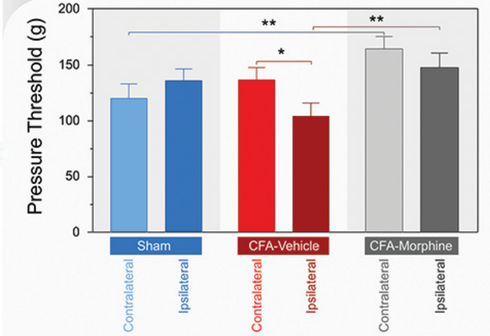
The graph shows a significant decrease in the mechanical pain threshold of the CFA-injected paw (ipsilateral side) as compared to the non-injected paw, suggesting the development of hyperalgesia in the affected paw. However, the threshold in sham animals progressively decreases with test repetition, suggesting that acquired pain avoidance reflex could be a potential bias to a repeated paw withdrawal measurement.

Figure 3: Spontaneous pain as reflected by weight bearing deficit



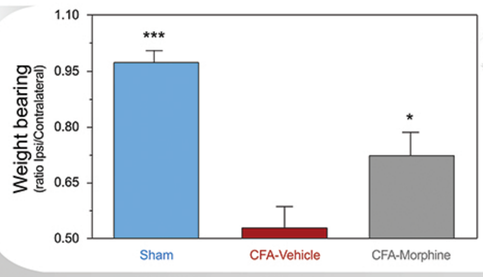
The graph shows the time course of hind limb weight bearing deficit (transfer of weight from the ipsilateral to the contralateral side. CFA injected rats as compared to the performance recorded in sham specimen, suggesting pain guarding behavior in the foot of CFA rats. The deficit (up 60% of weight transfer to the contralateral side) is maximal until day 4 but progressive recovery is observed from day 7.

Figure 4: Anti-nociceptive effect of Morphine against evoked pain



Graph showing the reversal by morphine of mechanical hyperalgesia observed in the CFA-injected paw. Noteworthy that Morphine significantly increases the mechanical threshold of the non-injected paw.

Figure 5: Anti-nociceptive effect of Morphine on spontaneous pain



Graph showing the attenuation of spontaneous pain by morphine treatment, as assessed by the change in the hindlimb weight bearing deficit of CFA-injected rats.

Key points:

- Response to evoked pain is affected by repetition bias
- Spontaneous pain as assessed by weight bearing deficit can be quantified in the rat model of monoarthritis
- Spontaneous pain is relieved by analgesic compound

Conclusion:

Measure of **weight bearing** deficit in the rat model of arthritis appears as an unbiased measure of pain and thus useful for pharmacological characterization of analgesic therapies.