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 (pedobarography) 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, 3, 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-Sellitlo algometer in the non-injected paw (contralateral side) and then in the CFA-injected paw (ipsilateral side).

The mechanical noxious 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 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.

[[Analgescic 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**

![Graph showing the time course of paw swelling after CFA injection. The swelling is measured at different timepoints after CFA injection (Days 0, 3, 4, 7, 10, 14, 21 and 28 post-CFA).](image)

**Figure 2: Evoked pain in response to mechanical hyperalgesia**

![Graph showing the time course of mechanical hyperalgesia after CFA injection. The hyperalgesia is measured at different timepoints after CFA injection (Days 0, 3, 4, 7, 10, 14, 21 and 28 post-CFA).](image)

**Figure 3: Spontaneous pain as reflected by weight bearing deficit**

![Graph showing the time course of weight bearing deficit after CFA injection. The deficit is measured at different timepoints after CFA injection (Days 0, 3, 4, 7, 10, 14, 21 and 28 post-CFA).](image)

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

![Graph showing the time course of weight bearing deficit after CFA injection. The deficit is measured at different timepoints after CFA injection (Days 0, 3, 4, 7, 10, 14, 21 and 28 post-CFA).](image)

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

![Graph showing the time course of weight bearing deficit after CFA injection. The deficit is measured at different timepoints after CFA injection (Days 0, 3, 4, 7, 10, 14, 21 and 28 post-CFA).](image)

[[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.