Croton oil-induced acute inflammation in mice

The protocol is designed to detect the inflammatory/anti-inflammatory potential effects of histamine ligands in a model of acute skin inflammation induced by local application of croton oil. The histamine H₁ receptor antagonist pyrilamine (30 mg/kg) and dexamethasone (2 mg/kg) represent the reference anti-inflammatory drugs administered subcutaneously immediately or 30 min before, respectively, the topic application of croton oil.

1. Animals preparation

- House CD-1 mice under controlled standard conditions (23±1°C, 55±10% humidity and a 12-h light/dark cycle). Provide standard laboratory chow and tap water ad libitum. Withhold food from animals for 18 hr, but allow free access to water, if the experimental protocol includes intragastric administrations.
- Weigh each mouse using an animal balance accurate to 1 g.
- Divide mice to receive randomly single doses of test compounds or the vehicle.

2. Croton oil-induced ear inflammation

- Carry out all experiments between 10 a.m. and 3 p.m., in order to avoid the influence of circadian variations in corticosteroid levels in the inflammatory responses.
- Dissolve test compounds in the appropriate vehicle and administer by subcutaneous (s.c., 3 ml/kg volume), intraperitoneal (i.p., 3 ml/kg volume) or intragastric (i.g., 10 ml/kg volume) route.
- Since the vehicle dimethyl sulfoxide (DMSO) reduces croton oil-induced edema (approximately 40%), dissolve test compounds in a vehicle containing 20% DMSO and 80% 2-hydroxypropyl-β-cyclodextrin, in amounts which do not change ear swelling per se.
- Administer vehicles or compounds under study, at increasing single doses, before the topic application of croton oil. Alternatively, when compound half-life is short, repeat the administration of test compound 2 hours after croton oil application.
- Induce cutaneous inflammation in conscious mice by topical application of croton oil (2.5% in acetone). Apply the irritant agent with a micropipette (20 µl/ear) to the inner surface of the right ear. Apply acetone to the left ear, which serves as a control (uninflamed ear).
- Two, 4 or 6 hours after croton oil application, kill the mouse by cervical dislocation and immediately remove both left (acetone) and right (croton oil in acetone) ears, by cutting horizontally across the indentation at the base of the ear.
- For each mouse, express the extent of the edema as the difference in weight (Δ mg) between right (inflamed) and left (uninflamed) ear.
- Express data as the means ± SEM from 6-8 mice per group.

3. Materials and reagents

- Male CD-1 mice, aged 6-8 weeks (20-25 g body weight)
- Animal balance accurate to 1 g (e.g. Sartorius)
- Analytical balance accurate to 0.1 mg
- Surgical instruments: sharp and blunt scissors, small straight anatomical forceps, large straight anatomical forceps
- 50-100 ml glass beakers
- 200-1000-5000 µl pipettes (e.g. Gilson)
- Magnetic stirrers
- Vortex mixers
- Croton oil (2.5% in acetone)
- Test compound solutions in the appropriate vehicle
- Prepare each compound immediately before use

References


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