Dehydroisohispanolone, a Selective NLRP3 Inflammasome Inhibitor with Therapeutic Potential in Gout

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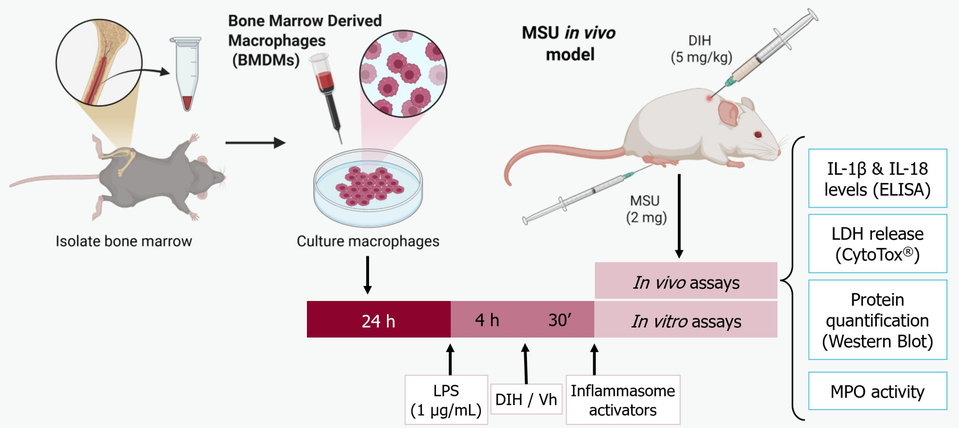
Introduction

Natural products, in particular terpenes, are a valuable source of molecules for drug discovery [1]. Hispanolone derivatives are bioactive diterpenes with broad therapeutic potential. Among them, Dehydroisohispanolone (DIH), a labdane-type diterpene, has shown the ability to modulate multiple inflammatory signaling pathways [2].

The inflammatory process is closely linked to the function of inflammasomes, which are intracellular multiprotein complexes also involved in the immune response. The NOD-like receptor protein 3 (NLRP3) inflammasome, a pyrin domain-containing a NOD-like receptor, is composed of three key components: the NLRP3 protein, procaspase-1, and the adaptor apoptosis-associated speck-like protein (ASC). Aberrant activation of NLRP3 inflammasome is involved in several inflammatory diseases, including gout.
This study explores the potential of DIH as a selective inhibitor of the NLRP3 inflammasome, with a specific focus on its applicability in treating gout-induced inflammation.

Materials and Methods



Results

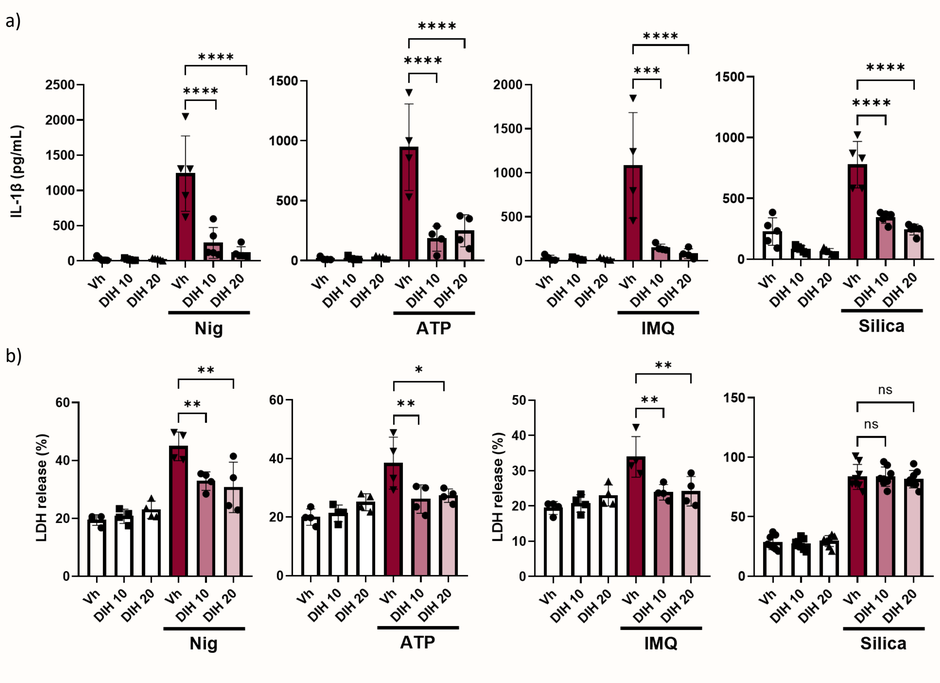


Fig. 1. DIH inhibits canonical NLRP3 inflammasome activation in primary macrophages. (a) LPS-primed (1 µg/mL, 4 h) BMDMs were preincubated with DIH (10, 20 µM) or Vh control for 30 min. Then, NLRP3 activator was added (Nig 10 µM, 1 h; ATP 5 mM, 1 h; Silica 300 µg/mL, 4 h or IMQ 75 µM, 1 h). Cell supernatants were analyzed for IL-1β released via ELISA. (b) Pyroptosis was assessed in supernatants using the CytoTox® kit. Mean ± SD (n=4-5). ns, no significant, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 *vs* LPS + stimuli treatment.

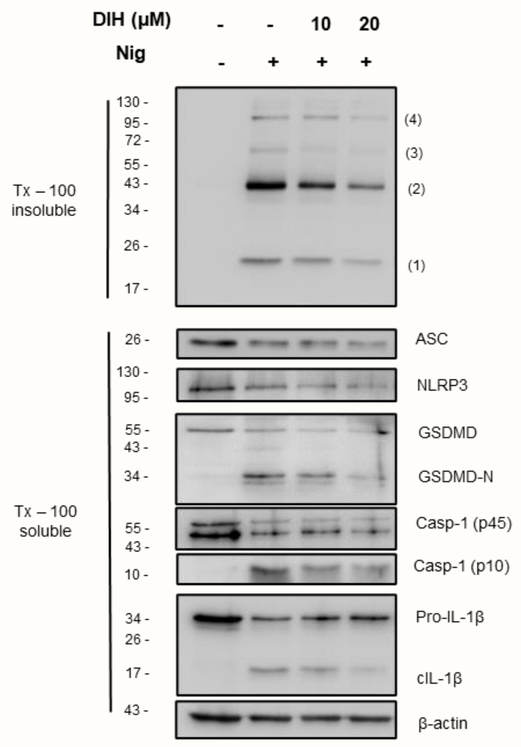


Fig 2. Suppression of inflammasome activation by DIH is dependent on ASC. LPS-primed (1 µg/ mL, 4 h) BMDMs were preincubated with DIH (10, 20 µM) for 30 min before stimulation with Nig (10 µM) for 1 h. Immunoblot analysis of crosslinked ASC oligomers in Triton x-100 insoluble pellet, and soluble total cell lysates probed with appropriated antibodies (ASC, NLRP3, GSDMD, caspase-1 and IL-1β). β-actin was used as a loading control. (n=4). **p < 0.005 and ***p < 0.001 *vs* LPS + Nig treatment.

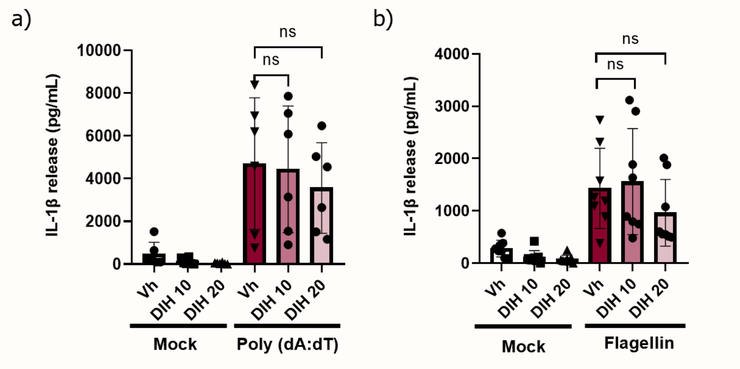


Fig 3. DIH does not affect activation of other inflammasomes (NLRC4 or AIM2). LPS-primed (1 µg/mL, 4 h) BMDMs were pretreated with diterpene DIH (10, 20 µM) or Vh for 30 min followed by stimulation with 1 µg/mL cytosolic poly (dA:dT) (a) or 1 µg/mL flagellin (b) for 4 h. Determination of IL-1β release was performed in cell supernatants. Mean ± SD (n=6). ns, no significant.

MSU-induced mouse gout model

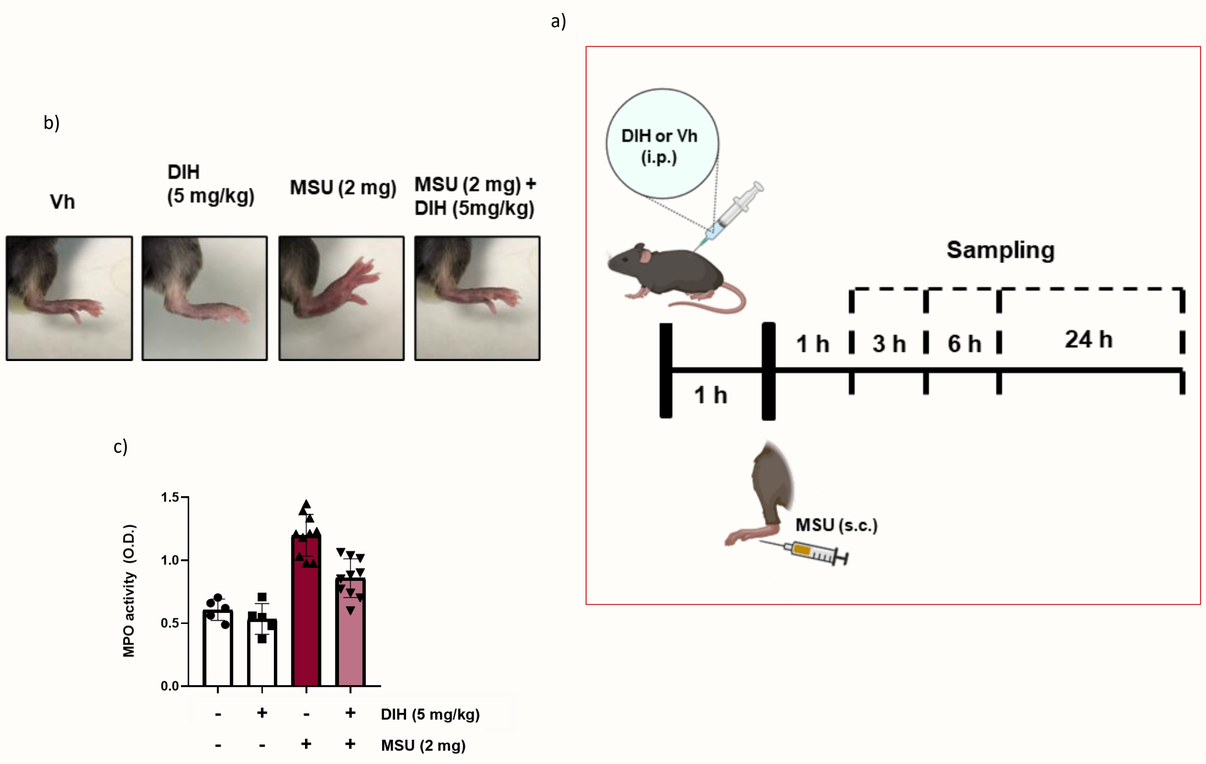


Fig 4. Pretreatment with DIH alleviates MSU-induced gouty arthritis in mice. DIH (5 mg/kg) or vehicle (Vh) were i.p. administered. After 1 h, MSU crystals (2 mg) were injected into the left hind paw. Mice were randomly divided into four groups (Vh and DIH: n=5/group; MSU and MSU + DIH: n=10/group). (b) Representative images of left hind paw at 24h post-MSU injection. (c) MPO activity was measured in paw homogenates at 24h (absorbance at 450 nm). Means ± SD. ****p<0.0001 MSU+DIH *vs* MSU.

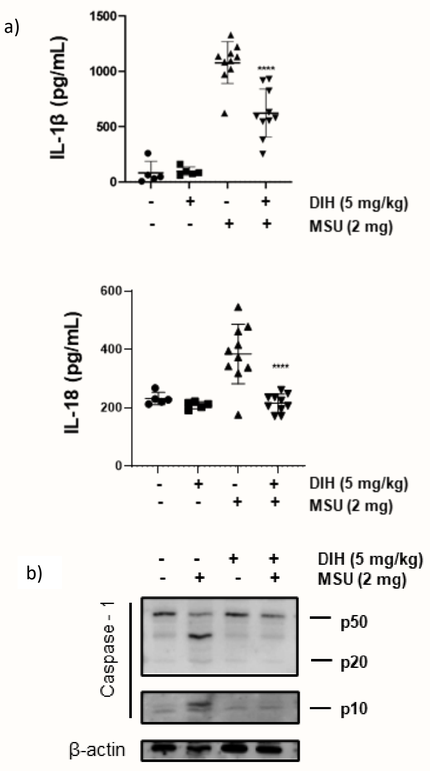


Fig 5. DIH inhibits the production of IL-1β and IL-18 in paw homogenates. DIH (5 mg/kg) or vehicle were i.p. administered. After 1 h, MSU crystals (2 mg) were injected into the left hind paw. (a) The levels of IL-1β and IL-18 were measured by ELISA. Mean ± SD of n=5 mice in Vh (-) and DIH groups and n=10 mice in MSU and MSU + DIH groups. (b) Immunoblotting for Caspase-1. β-actin was used as a loading control (n=4). ****p<0.0001 MSU+DIH *vs* MSU.

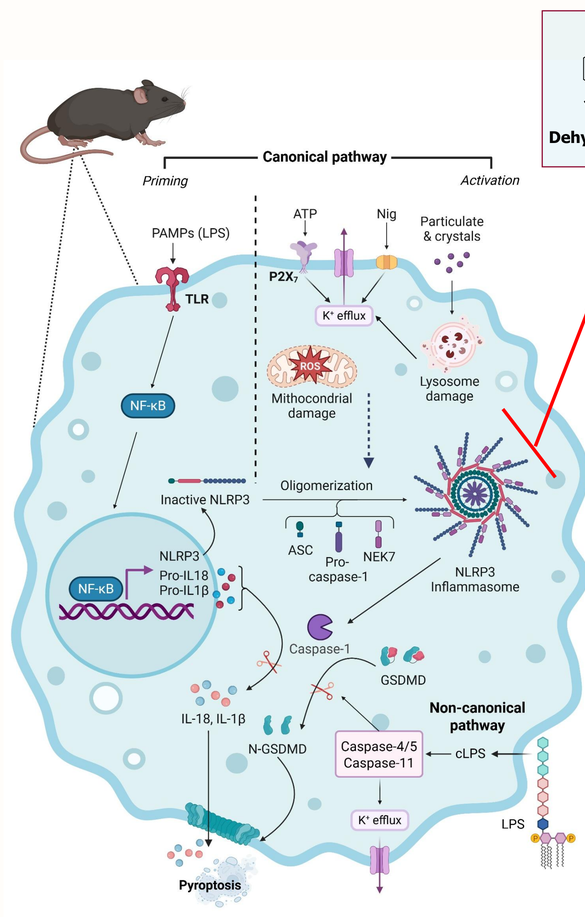


Fig 6. Illustration of the proposed molecular inhibitory mechanisms of DIH in the NLRP3 inflammasome pathway.

Conclusions

1. DIH significantly inhibited canonical NLRP3 inflammasome activation, reducing IL-1β release in response to a different stimuli: Nig, ATP, IMQ and silica in LPS-primed bone marrow macrophages.
2. This diterpene interfered with the assembly of the NLRP3 inflammasome complex, which is likely achieved by targeting ASC oligomerization, with no effect on AIM2 or NLRC4 inflammasomes.
3. DIH effectively mitigated acute gout by blocking NLRP3 inflammasome activation, reducing MSU-induced swelling, neutrophil infiltration.
4. The diterpene DIH may be a novel therapeutic agent targeting NLRP3 for the treatment of acute gouty based on its anti-inflammatory properties.

References