

Evaluating the *in vivo* efficacy of NLRP3 inhibitor, NT-0249, in pre-clinical models of chronic joint pain

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Background

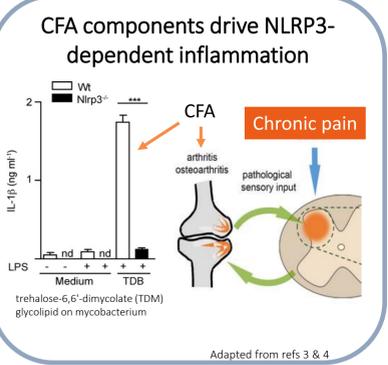
Chronic pain remains an unmet clinical need, affecting over 25% of the US population. Inflammasomes, critical components of the immune system, control cytokine release, which in turn modulates sensory neuron function. The NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome in particular, has been implicated in the initiation and maintenance of chronic pain states and may represent a novel druggable target for the treatment of pain.

NT-0249, a potent, selective, brain-penetrant NLRP3 inhibitor, currently in clinical development, has demonstrated peripheral and central anti-inflammatory effects in diverse pre-clinical models, including peritonitis, cryopyrin-associated periodic syndrome (CAPS) and diet-induced obesity (1,2).

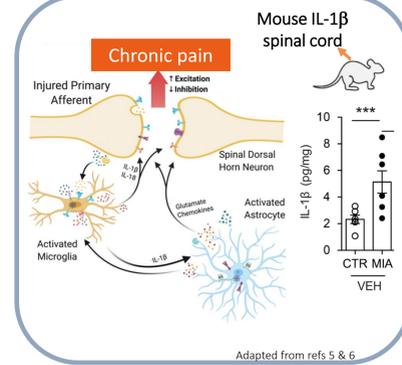
Methods

To evaluate the therapeutic potential of NT-0249 in pain, we employed a multi-step approach involving *in vitro*, pharmacokinetic, and *in vivo* efficacy studies. Human iPSC-derived microglial cultures were used to assess NT-0249 potency by measuring its effects on NLRP3-dependent IL-1 β release. CSF/brain exposures were assessed in rats using intracerebral microdialysis. The *in vivo* efficacy of NT-0249 was subsequently tested in mouse models of Complete Freund's adjuvant (CFA) or monoiodoacetate (MIA)-induced mechanical hypersensitivity.

CFA model

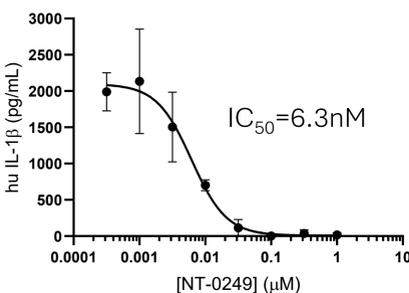
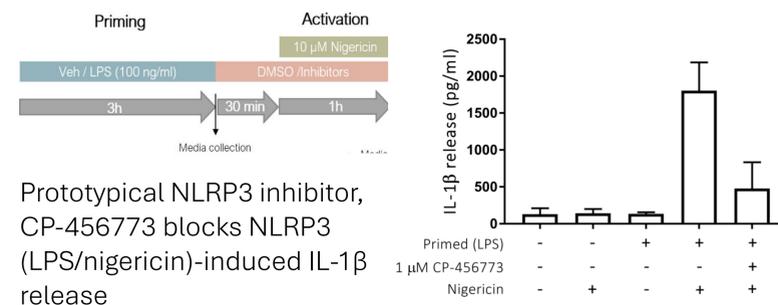


MIA model



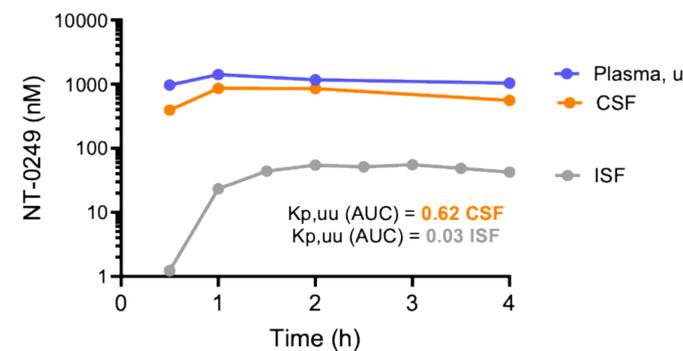
Results

iPSC-derived human microglial cultures secrete IL-1 β in response to NLRP3 activation



Pre-treatment with NT-0249 fully blocks NLRP3-driven IL-1 β release from human iPSC microglial cultures with high potency.

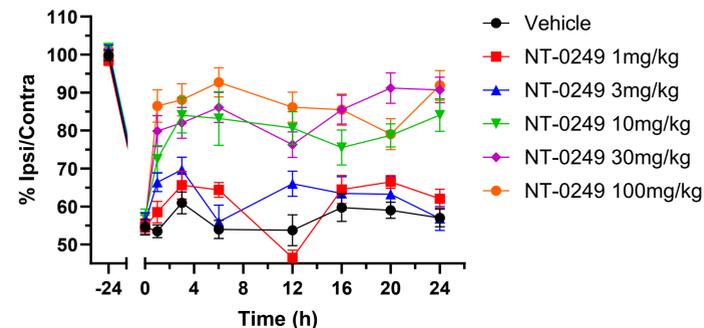
Microdialysis in the rat demonstrates CSF and brain penetration of NT-0249 following oral dosing



NT-0249 orally dosed in rat (10 mg/kg), showed rapid distribution to CSF and brain penetration to levels above IC_{50} .

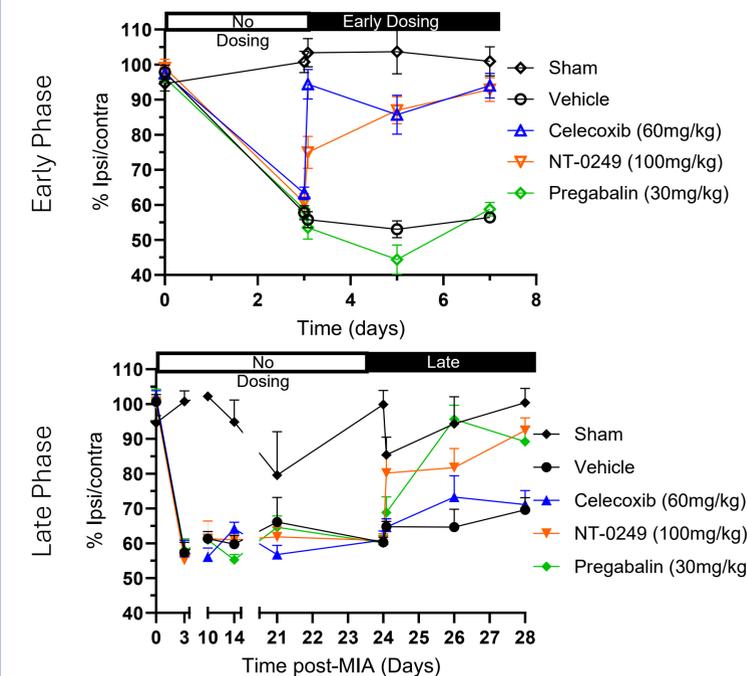
Results

Weight bearing efficacy in CFA joint inflammation model



NT-0249 reversed mechanical joint hypersensitivity at 10-100mg/kg, while doses <10 mg/kg provided limited benefit.

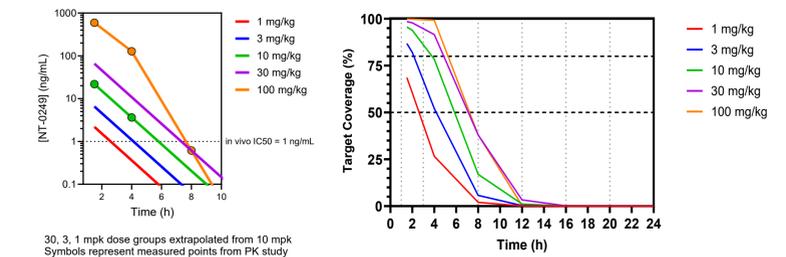
Weight bearing efficacy in intra-articular MIA model



NT-0249 demonstrated significant efficacy in early and late phase of intra-articular MIA-induced mechanical allodynia. Robust reversal of mechanical hypersensitivity is observed with NT-0249.

Results

NT-0249 – peripheral exposure and modelled target cover

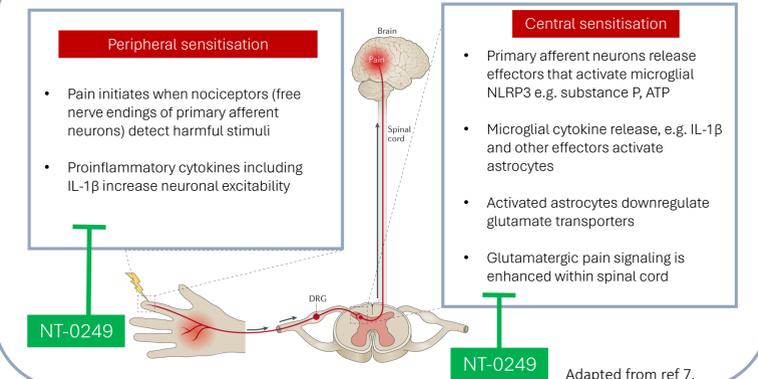


Exposure-target cover relationships indicate that doses of 10-30 mg/kg achieve robust NLRP3 inhibition.

Conclusions

- NT-0249 is a potent inhibitor of NLRP3
- NT-0249 inhibits IL-1 β release from human microglia
- NT-0249 is CNS penetrant
- NT-0249 reverses hypersensitivity as demonstrated in two orthogonal chronic joint inflammation models in mouse

NT-0249 blocks nociceptive signaling at multiple levels



References

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