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A nanomedicine approach for diabetes: targeting the NLRP3 inflammasome in tissue-resident macrophages.

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Background

Type 2 diabetes: chronically elevated blood glucose levels caused by insufficient insulin levels, due to…

Beta-cell dysfunction
- Progressive loss of functional insulin-producing beta cells of the pancreas

Insulin resistance
- Diminished insulin-receptor signalling in tissues including adipose, liver, skeletal muscle

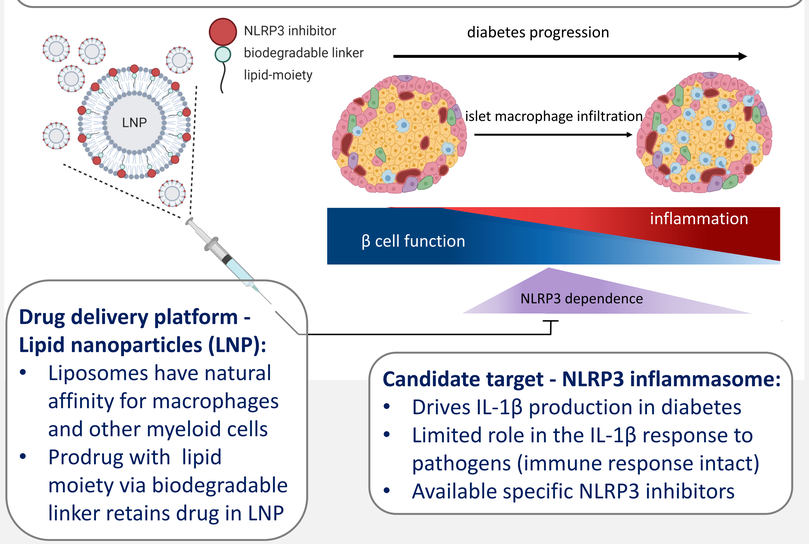
Inflammation: drives beta-cell dysfunction and insulin resistance
- Adipose tissue and pancreatic islets infiltrated by macrophages
- NLRP3 inflammasome (in macrophages) drives production of IL-1β
- IL-1β impairs insulin secretion and signalling

Problem

Anti-inflammatory drugs as potential therapeutics for diabetes are limited by a) off-target effects and toxicity, and b) systemic immune suppression.

Goal

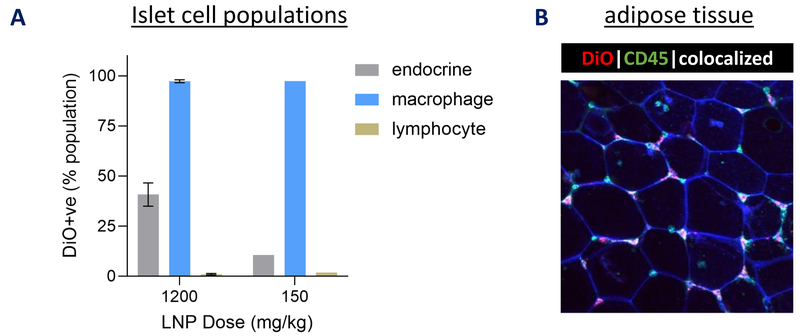
Deliver **anti-inflammatory** drugs **specifically to macrophages** (including those in islets and adipose) to **treat diabetes** while limiting toxicity and immune suppression



Drug delivery platform - Lipid nanoparticles (LNP):
- Liposomes have natural affinity for macrophages and other myeloid cells
- Prodrug with lipid moiety via biodegradable linker retains drug in LNP

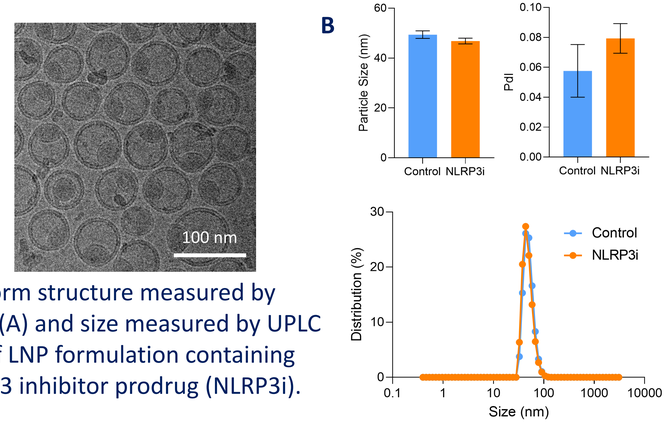
Candidate target - NLRP3 inflammasome:
- Drives IL-1β production in diabetes
- Limited role in the IL-1β response to pathogens (immune response intact)
- Available specific NLRP3 inhibitors

LNP formulation selectively targets macrophages



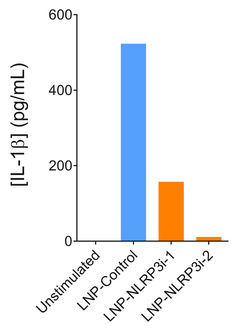
Uptake of DiO-labelled LNPs in islet cells measured by flow cytometry (A) and adipose cells measured by histology (B, CD45=immune cell marker).

NLRP3i-LNP characteristics



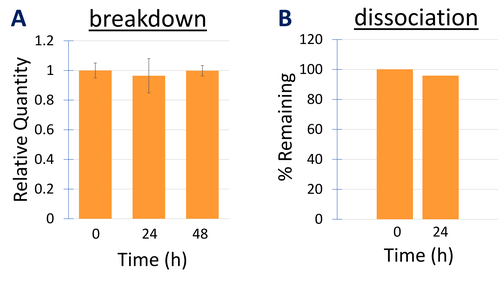
Uniform structure measured by TEM (A) and size measured by UPLC (B) of LNP formulation containing NLRP3 inhibitor prodrug (NLRP3i).

NLRP3i-LNPs inhibit NLRP3 activation in macrophages



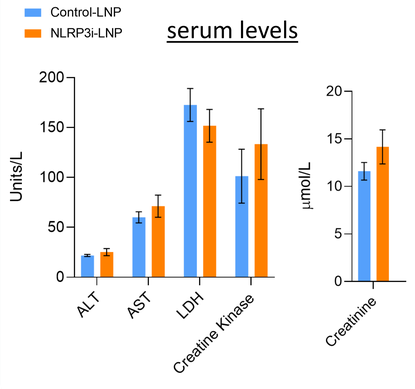
Efficacy of 2 lead NLRP3 inhibitors formulated at 20 mol% in LNP compared to LNP-control. Inhibition of NLRP3 was measured by IL-1β secretion from LPS+ATP stimulated mouse bone marrow derived macrophages. Unstimulated controls show lack of IL-1β secretion without LPS+ATP activation.

LNPs effectively retain NLRP3i



Breakdown of prodrug (A) (relative quantity of prodrug to initial) of LNP-encapsulated NLRP3i over time in mouse plasma measured by UPLC. Dissociation of NLRP3i from LNP (B) (percent of prodrug remaining in LNP) over time in human plasma measured by UPLC.

Chronic NLRP3i-LNP treatment is non-toxic



Markers of liver toxicity (ALT, AST), general toxicity (LDH), muscle toxicity (Creatinine Kinase) and renal toxicity (Creatinine) in NLRP3i-LNP (40 mg/kg) and control-LNP treated mice (injected i.p. 2x weekly for 15 weeks).

Conclusions

- LNP formulations specifically and efficiently target macrophages, including those in islets and adipose tissue
- NLRP3i-LNPs:
  - Inhibit IL-1β release from macrophages
  - Do not display toxicity after long-term administration in mice

Future Directions

Test efficacy of NLRP3i-LNP to in mouse models of diabetes

Acknowledgements

BC Children's Hospital — An agency of the Provincial Health Services Authority
UBC
Integrated nanotherapeutics
Michael Smith Foundation for Health Research — BC's health research funding agency
NMIN — Nanomedicines Innovation Network / Réseau d'innovation nanomédecines
JDRF — Improving lives. Curing Type 1 diabetes.