Liposomal prednisolone promotes macrophage necroptosis in experimental atherosclerosis: *does this explain atherogenesis in Cushing´s disease?*

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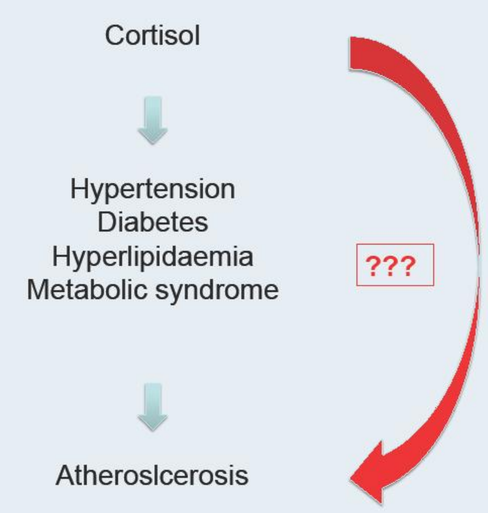
EP195

Rationale: Liposomal nanoparticles loaded with prednisolone phosphate (LN-PLP) have previously been reported to accumulate in macrophages of rabbits' atherosclerotic lesions, and rapidly reduce arterial wall inflammation. In patients with atherosclerotic disease, accumulation of LN-PLP in macrophages of atherosclerotic plaques has been demonstrated, but arterial wall inflammation reduction was not observed.

Objective: To evaluate the effect of LN-PLP´s effect on inflammatory macrophages in a mouse model of atherosclerosis.

Methods and Results: In low-density lipoprotein receptor knockout ($LDLR^{-/-}$) mice on high fat diet, we show that LN-PLP accumulates in plaque macrophages and biweekly injections at 10mg/kg induces (i) enhanced monocyte recruitment to the plaque, leading to (ii) increased macrophage content, more advanced plaque stages, and larger necrotic core sizes after 6 weeks of treatment. *In vitro*, we observed that both murine and human macrophages polarize into a lipophilic phenotype following LN-PLP exposure, illustrated by increased lipid accumulation, endoplasmatic reticulum (ER) stress and necroptosis.

Conclusion: These findings indicate that local exposure to the anti-inflammatory compound prednisolone, can elicit a pro-atherogenic, lipotoxic effect in plaque macrophages. This might explain atherogenesis in patients with Cushing´s disease.



Efficacy: monocyte recruitment

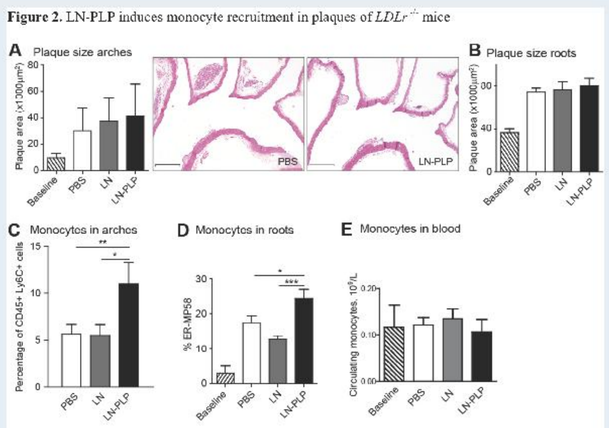


Figure 2. LN-PLP induces monocyte recruitment in plaques of $LDLr^{-/-}$ mice

(**A,B**) Plaque size was quantified in (**A**) the aortic arches (original magnification x25; scale bars represent 1mm) and (**B**) the roots using Hematoxylin-Eosin (HE) staining, 6 weeks after HFD (baseline, n=6) and an additional 2 weeks with PBS, LN or LN-PLP iv administration (n=20/group, 10mg/kg, biweekly). (**C**) Pro-inflammatory monocytes were assessed by flow cytometry of the arches, and (**D**) % of the lesion positive for ER-MP58 staining, indicative of freshly recruited monocytes in the roots, (**E**) the number of blood monocytes. Data are presented as mean ± SEM; *P<0.05, **P<0.01. LN = empty liposomes, LN-PLP = liposomal prednisolone, PBS = phosphate-buffered saline.

Efficacy: gene expression

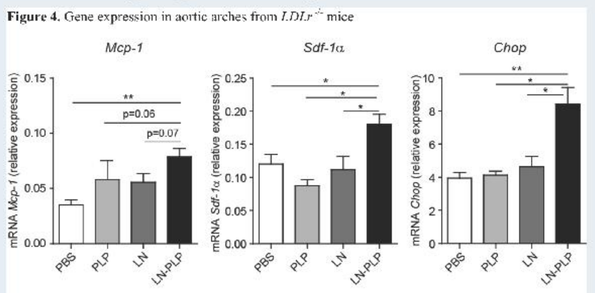


Figure 4. Gene expression in aortic arches from $LDLr^{-/-}$ mice

mRNA was isolated from aortic arches of $LDLr^{-/-}$ mice after 6 weeks administration of either PBS, PLP, LN or LN-PLP (n=16, 10mg/kg iv, biweekly) to analyse gene expression of monocyte migration factors, *Mcp-1* and *Sdf-1α*, and the endoplasmatic reticulum stress marker, *Chop*. Gene expression was normalized to *36B4* housekeeping gene. Data are presented as mean ± SEM; *P<0.05, **P<0.01. *Chop* = C/EBP homologous protein, LN = empty liposomes, LN-PLP = liposomal prednisolone, *Mcp-1* = monocyte chemotactic protein-1, PBS = phosphate-buffered saline, PLP = free prednisolone, *Sdf-1α* = stromal cell-derived factor-1α.

Delivery

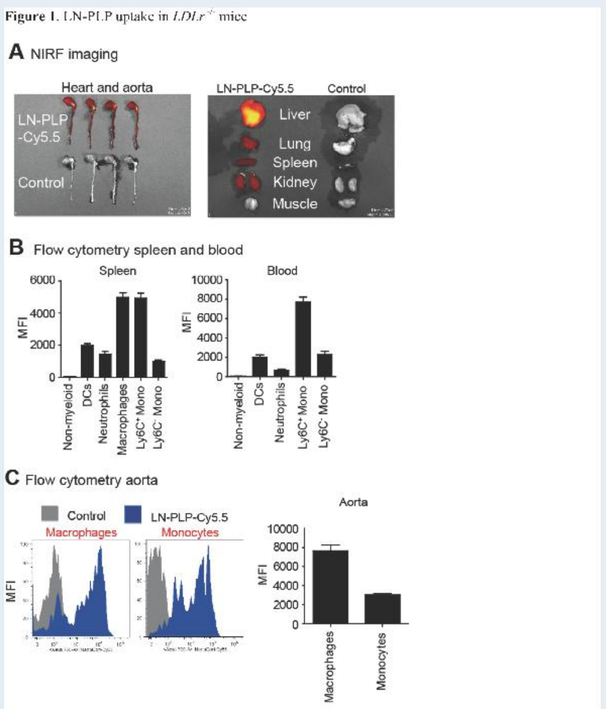


Figure 1. LN-PLP uptake in $LDLr^{-/-}$ mice

(**A**) NIRF images showing accumulation and distribution of Cy5.5-labelled LN-PLP (n=4) or PBS (n=4) 24 hours after tail vein injection (10mg/kg) in 8-week old $LDLr^{-/-}$ mice, 6 weeks on HFD. (**B,C**) Cellular distribution of LN-PLP in spleen, blood and aorta was assessed by flow cytometry. Data are presented as mean + SEM. HFD = high fat diet, LN-PLP = liposomal prednisolone, NIRF = near infrared fluorescence, PBS = phosphate-buffered saline.

Efficacy: plaque size

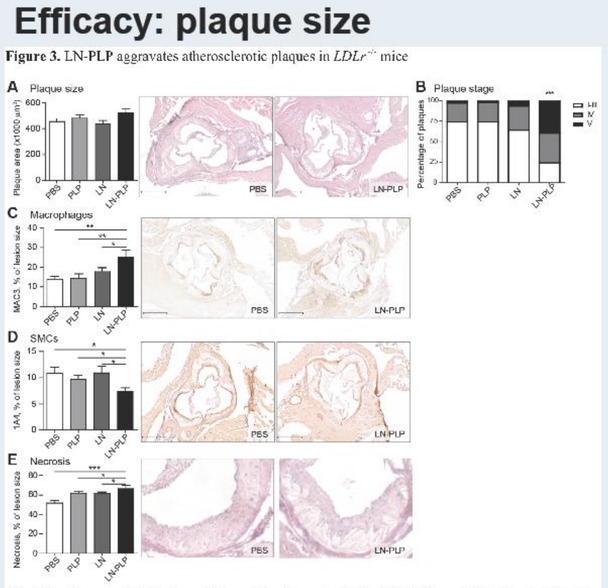


Figure 3. LN-PLP aggravates atherosclerotic plaques in $LDLr^{-/-}$ mice

(**A**) After 6 weeks HFD alone, followed by 6 weeks PBS, PLP, LN or LN-PLP administration (n=16/group, 10mg/kg iv, biweekly), (**A**) plaque size and (**B**) plaque stage were determined in HE stained sections of the roots. (**C-E**) Representative photomicrographs and quantifications (**C**) of macrophage content (MAC-3), (**D**) smooth muscle cell content (1A4) and (**E**) necrotic core size are shown. **A,C,D** original magnification x25; **E**, x100; scale bars represent 1mm. Data are presented as mean±SEM; *P≤0.05, **P≤0.01, ***P≤0.001. LN = empty liposomes, PLP = free prednisolone, LN-PLP = liposomal prednisolone, PBS = phosphate-buffered saline.

in vitro: lipotoxicity and necroptose

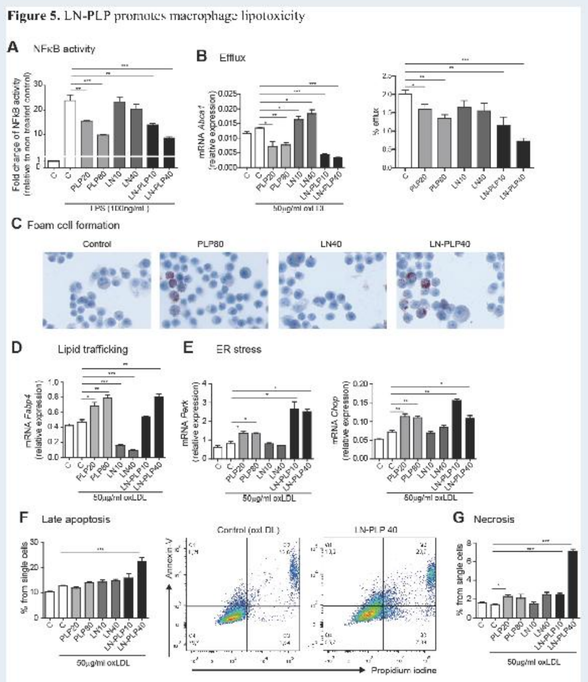


Figure 5. LN-PLP promotes macrophage lipotoxicity

(**A**) NFκB activity was determined in RAW267.4 NFκB-*luc* cells as the relative fold change to control without 100ng/ml LPS stimulation. (**B**) BMDM were isolated from C57BL/6 mice to study gene expression levels of ATP-binding cassette transporter A1 (*Abca1*) and cholesterol efflux towards the acceptor apo-A1. (**C**) BMDM lipid content after 48 hours oxLDL (50 ug/ml) using oil-red-O stain. (**D**) Gene expressions of the intracellular lipid transporter *Fabp4*, and (**E**) ER-stress markers *Perk* and *Chop* in BMDM exposed to oxLDL (50 ug/ml) for 24 hours. (**F-G**) Late apoptosis and necrosis

Wissen schafft Gesundheit

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