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Prednisolone-containing liposomes accumulate in human atherosclerotic macrophages upon intravenous administration

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Abstract

Drug delivery to atherosclerotic plaques via liposomal nanoparticles may improve therapeutic agents' risk–benefit ratios. Our paper details the first clinical studies of a liposomal nanoparticle encapsulating prednisolone (LN-PLP) in atherosclerosis. First, PLP's liposomal encapsulation improved its pharmacokinetic profile in humans (n = 13) as attested by an increased plasma half-life of 63 h (LN-PLP 1.5 mg/kg). Second, intravenously infused LN-PLP appeared in 75% of the macrophages isolated from iliofemoral plaques of patients (n = 14) referred for vascular

Abbreviations: FDG-PET/CT, ^{18}fluorodeoxyglucose positron emission tomography/computer tomography; BMI, body mass index; CVD, cardiovascular disease; DCE-MRI, dynamic contrast enhanced-magnetic resonance imaging; DPPC, dipalmitoylphosphatidylcholine; DSPE, distearoylphosphatidylethanola-mine; GC, glucocorticoids; HDL-C, high density lipoprotein cholesterol; LCA, left carotid artery; LDL-C, low density lipoprotein cholesterol; LN, liposomal nanoparticle; LN-PLP, liposomal nanoparticle encapsulating prednisolone phosphate; MPS, mononuclear phagocyte system; PEG, polyethylene glycol; PLP, prednisolone phosphate (prodrug); PL, prednisolone (free drug); RCA, right carotid artery; ROI, region of interest; SBP, systolic blood pressure; SUV, standardized uptake value; TBR, target to background ratio; TC, total cholesterol; TG, triglycerides; VWA, vessel wall area.

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surgery in a randomized, placebo-controlled trial. LN-PLP treatment did however not reduce arterial wall permeability or inflammation in patients with atherosclerotic disease (n = 30), as assessed by multimodal imaging in a subsequent randomized, placebo-controlled study. In conclusion, we successfully delivered a long-circulating nanoparticle to atherosclerotic plaque macrophages in patients, whereas prednisolone accumulation in atherosclerotic lesions had no anti-inflammatory effect. Nonetheless, the present study provides guidance for development and imaging-assisted evaluation of future nanomedicine in atherosclerosis.

Key words: Atherosclerosis; Nanomedicine; Glucocorticoids; Macrophages

Background

Because inflammation plays a pivotal role in atherosclerotic plaque development,[1] novel anti-inflammatory strategies[2] are expected to complement and improve existing therapeutic regimens. Delivering drugs via nanocarriers may reduce atherosclerotic plaque inflammation by enhancing drug accumulation at target sites, without compromising immunity.[3] Though several liposomally formulated anticancer drugs have already been approved for clinical use,[4] nanomedicine remains unexplored in patients with cardiovascular disease. Theoretically, an inflamed atherosclerotic plaque, characterized by endothelial dysfunction and a highly permeable microvasculature, could be an excellent target for nanomedicinal delivery.[5]

Of the numerous clinically-applied anti-inflammatory compounds, glucocorticoids (GCs) are the most widely used and have potent anti-inflammatory effects.[6] However, systemic GC treatment has not been used in patients with cardiovascular disease because long-term GC use has pro-atherogenic effects, including dyslipidemia, glucose intolerance and hypertension.[7] In contrast, locally administering GCs via drug-eluting stents has been shown to reduce neo-intimal formation and arterial wall inflammation in an experimental model.[8] These results suggest that a liposomal GC formulation may minimize systemic adverse effects while improving local anti-inflammatory efficacy. In support of this idea, we previously reported markedly reduced arterial wall inflammation following intravenous administration of liposomal prednisolone in an atherosclerotic rabbit model.[9]

Here we evaluate the clinical applicability of a long-circulating liposomal nanoparticle encapsulating prednisolone phosphate (LN-PLP) in patients with atherosclerosis. First, we determined liposomal prednisolone's pharmacokinetic profile in humans and assessed delivery to plaque macrophages isolated from iliofemoral plaques of patients referred for vascular surgery. Subsequently, we used noninvasive multimodal imaging to measure the anti-inflammatory efficacy of LN-PLP in patients with atherosclerosis.

Methods

Study participants

All participants provided written informed consent. The clinical trials were approved by the local institutional review board and conducted according to the principles of the International Conference on Harmonisation–Good Clinical Practice guidelines (Clinicaltrials.gov registration NCT01039103, NCT01647685, NCT01601106).

Liposomal prednisolone

The liposomal nanoparticles (LNs) were composed of a hydrophilic core encapsulating prednisolone phosphate (PLP), surrounded by a lipid bilayer of phospholipids and cholesterol, which was coated with polyethylene glycol (PEG). See supplementary methods for LN-PLP formulation.

Pharmacokinetic profile of LN-PLP in humans

We conducted a single-dose escalation study, using 13 subjects, to determine the pharmacokinetic performance of LN-PLP after a single intravenous (i.v.) dose of 0.375 mg/kg (n = 3), 0.75 mg/kg (n = 3) or 1.5 mg/kg (n = 7) LN-PLP in a 2.5 h time frame. Serum concentrations of PL and its pro-drug PLP were measured on days 1, 3, 7 and weekly up to 12 weeks using high-performance liquid chromatography. Safety evaluation after LN-PLP administration included documenting adverse events, checking vital signs and conducing safety laboratory tests.

LN-PLP delivery in patients with iliofemoral atherosclerosis

To study LN-PLP's delivery, we performed a randomized, placebo-controlled, double-blind trial in 14 patients with iliofemoral atherosclerotic plaques who were scheduled for endarterectomy. After 1:1 randomization, patients received either 1.5 mg/kg LN-PLP (n = 7) or saline (n = 7) via an antecubital vein on days 0 and 7, followed by vascular surgery on day 10. The dosing regimen for LN-PLP was based on a preclinical study in rabbits[9] and adjusted according to the drug-dose conversion from rabbit to human.[10]

Plaque tissue macrophages were isolated to evaluate the presence of LN-PLP (see supplementary methods)[11]. Cells were spotted on a glass slide using a cytospin centrifuge, fixed with 0.4% paraformaldehyde (30 min), permeabilized with 0.1% Triton X-100 (10 min) and stained overnight. Primary antibodies were mouse anti-human CD68 (Abcam, Cambridge, UK; 1:100) and rabbit anti-human PEG (Epitomics, Burlingame, CA, U.S.A.; 1:100), and secondary antibodies were CyTM3-conjugated donkey anti-mouse and FITC-conjugated donkey anti-rabbit (both Jackson, West Grove, PA, 1:200). Cells were examined with fluorescence microscopy (Leica, DMRA HC Upright). Per patient, at least 4 cell spin slides were used to examine the percentage of DAPI cells positive for CD68 (macrophages) and, DAPI/CD68 cells positive for PEG (LN-PLP). A reader who was blinded for treatment allocation performed these analyses.

Local efficacy in patients with atherosclerosis

Subsequently, we evaluated LN-PLP therapeutic efficacy in a randomized, placebo-controlled, double-blind trial of 30 patients with documented history of atherosclerotic cardiovascular disease (i.e. angina pectoris, myocardial