Supplements

Supplementary Methods

Manufacturing of liposomal prednisolone

Pegylated liposomal prednisolone consists of small (100 nm) phospholipid vesicles coated with PEG. The lipid bilayer encloses an aqueous compartment in which the water-soluble disodium phosphate derivative of prednisolone is entrapped. Each mL formulation contains 2.8 mg/mL prednisolone sodium phosphate, 30 mg palmitoyl phosphatidyl choline (DPPC), 9 mg distearoyl phosphatidyl ethanolamine-PEG2000 (PEG-DSPE), and 8 mg cholesterol. The liposomes are dispersed in 10% sucrose buffered with phosphate buffer at a pH of 7.4.

The product was prepared by mixing an alcoholic solution of the lipid constituents with an aqueous solution of the corticosteroid followed by repeated high-shear homogenization to reduce the size of the formed vesicles. Unencapsulated prednisolone phosphate was removed by ultrafiltration and washing with dispersion buffer. The process was concluded by sterile filtration using 0.2 micrometer filter membranes and filling. The production of GMP-grade liposomes resulted in formulations with a size ranging from 90 to 110 nm (mean size: 100 nm ± 10 nm), with a polydispersity index of <0.1. The prednisolone phosphate incorporation efficiency was between 3-5%. The zeta potential was consistently – 5 mV. The final prednisolone phosphate content varied between 1.0 and 3.2 mg/mL. Free drug content was always below 5% of the total PLP content. The phospholipid content varied between 22 mg/mL and 47 mg/mL. Both drug and lipid content were measured by validated HPLC assays. Also sterility and pyrogenicity were measured (the latter determined with the LAL assay (Biowhittaker, Walkersville, MD), as was the residual content of alcohol (gas chromatography, specification <0.1%). All raw material purchased was GMP-certified and the liposome manufacturing was performed under GMP conditions.

Manufacturing of fluorescent liposomes

Fluorescent liposomes were manufactured in a non-GMP setting, via a similar process as liposomal prednisolone. These liposomes were loaded with Cy5.5 instead of prednisolone.

Kidney transplantation protocol

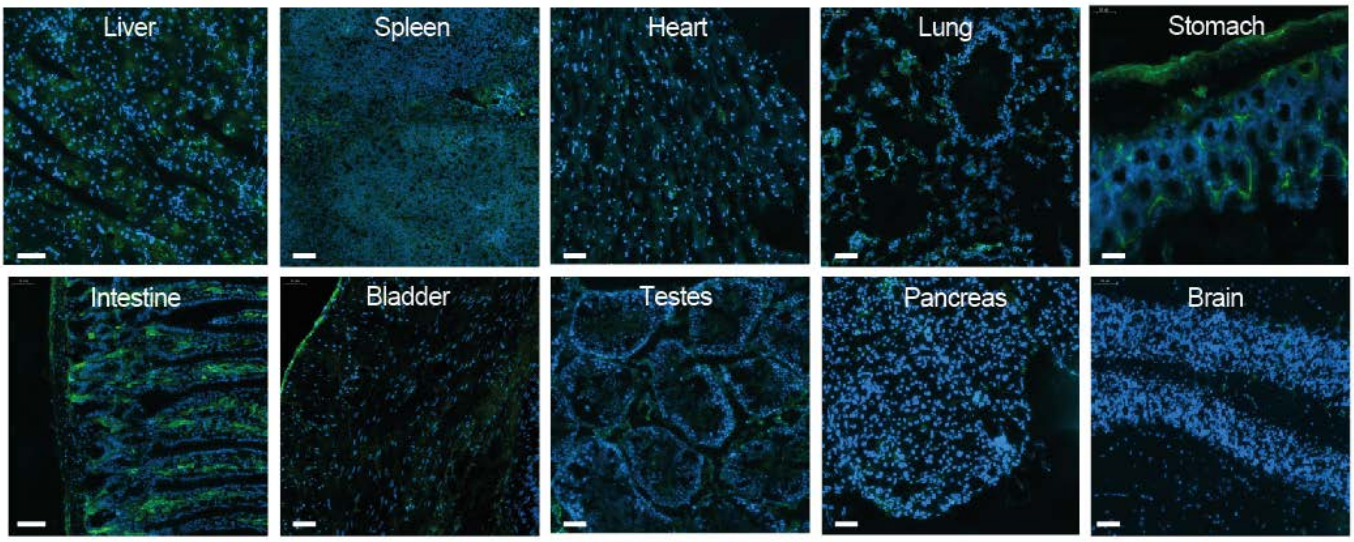
Briefly, surgeries were performed under isoflurane anesthesia (5% induction, 2-3% maintenance) with butorphanol for analgesia. After midline incision and dissection of the left donor kidney, the whole animal was flushed with ice cold PBS via the left ventricle to remove the blood. The left donor kidney was removed en bloc with the renal artery and vein and the ureter and stored at 4°C for 60 minutes to standardize cold ischemia time. Next, the left kidney from the BALB/c recipient was removed and the

graft was placed in the lower abdomen. Vascular anastomoses were made and the ureter was sutured into the bladder dome during 30 min warm ischemia time. The contralateral kidney was left in place.

MRI

For renal perfusion quantification a respiratory-triggered, fat-saturates flow-alternating inversion (FLAIR) recovery arterial spin labeling (ASL)-sequence with an echo-planar readout was performed in a central coronal plane by using the following scan parameters: TR/TE = 18.000/16.4 msec, 13 inversion times = 30, 100, 200, 300, 500, 700, 1000, 1200, 1500, 2000, 3000, 5000 and 8000 msec, matrix = 128 x 128, field of view = 35 x 35 mm2, slice thickness = 2mm. To avoid respiratory motion ASL-images were co-registered and quantitative perfusion data were calculated using MatLab software (v. 7.11.0.584, MathWorks, Natick, MA) as described previously [27]. Regions of interest (ROI) were placed manually into the renal cortex.

Supplementary Figures



Supplementary figure 1. Cy5.5^{+} liposome localization in recipients organs. Representative images of recipients nontarget organs show little to none uptake of fluorescent liposomes. Images are shown in a 300x magnification with a scale bar of 50 µm. An anti-PEG antibody was used to visualize quenched Cy5.5^{+} liposomes (green), and sections were sealed with Prolong Gold antifade mountant with DAPI (blue).

Supplementary Tables

Table 1 Primer sequences used to quantify mRNA expression in mouse tissue

Target	Forward primer 5' to 3'	Reverse primer 5' to 3'
Fkbp5	GCCGACTGTGTGTGTAATGC	CACAATACGCACTTGGGAGA
Il6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
Ccl2	GCACCAGCCAACTCTCAC	CTTCTTGGGGTCAGCACAG
Tnfa	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Cxcl2	CCAGACAGAAGTCATAGCCACT	CTTCCGTTGAGGGACAGCAG
Il1b	GCTACCTGTGTCTTTCCC	TCGTTGCTTGGTTCTCCTTG
Il10	GCTGGACAACATACTGCTAACC	CCCAAGTAACCCTTAAAGTCCTG
Cd45	GTTTTCGCTACATGACTGCACA	AGGTTGTCCAACTGACATCTTTC
Trap5b	AACCTGCAGTATCTTCAGGACG	TCGTTGATGTCGCACAGAGG
Actin	AGGTCATCACTATTGGCAACGA	CCAAGAAGGAAGGCTGGAAAA

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Table 2 Onset of side effects after treatment with NA, P, or LP. Data is presented as group mean ±SD. NA= no additional treatment, P=Prednisolone, LP = liposomal prednisolone, n.s. indicates nonsignificant findings.

	NA	P	LP	p-value
Plasma glucose day 0 (µmol/l)	7.3±1	6.7±1	7.3±1	n.s.
Plasma glucose day 7 (µmol/l)	5.8±1	5.7±2	6.5±2	n.s.
Plasma insulin (mU/l)	30.0±32	24.0±12	58.9±60	n.s.
Brown adipose tissue activation (area% of white fat)	8.8±7	13.2±7	14.9±6	n.s.
Liver weight (mg)	1668±491	1607±239	1426±461	n.s.
Total liver cholesterol (nmol/mg tissue)	6.4±1	6.6±1	7.0±2	n.s.
Spleen weight (mg)	282.5±89	220.0±50	146.3±116	LP vs NA, p=0.04