In vivo and in vitro biodistribution of solid lipid nanoparticles

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Introduction: Solid lipid nanoparticles (SLN) are a versatile tool with a high potential of applications. They are constituted by biocompatible and biodegradable matrix with well-established safety profiles. Their matrix can improve the stability and bioavailability of labile molecules, assuring restrained release profile. Moreover, due to the physicochemical properties of lipids (i.e. the low melting temperature), SLN can be easily obtained by direct emulsification of the molten lipids and subsequent recrystallization, thus avoiding the use of potentially toxic solvents that are commonly required for the preparation of polymeric nanoparticles (1). SLN used for this study were produced by a protocol based on emulsification of the molten lipids in water by melt and ultra-sonication method (2), and were functionalized with polysorbate 80 (SLN-P80) for in vivo administration. In fact, it has been reported that this surfactant extend the nanoparticle circulation time in the blood, avoiding opsonization with the complement activation and uptake by the reticulo-endothelial system. The influence of nanoparticle composition and functionalization was investigated on morphology, dimension and inner structure by mean of cryogenic transmission electron microscopy (cryo-TEM), X-ray diffraction measurements, photon correlation spectroscopy (PCS) and sedimentation field flow fractionation (SdFFF) (2).

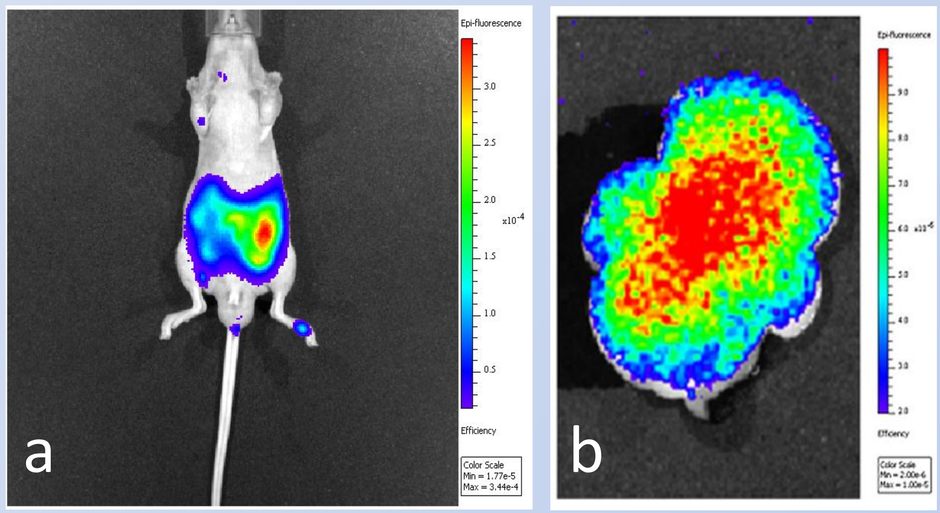

Figure 1. FLI, (a), ventral acquisition of a representative nude mouse after 4h from SLN IP administration, b) liver explanted from a mouse 4h after SLN IP administration and perfusion.

In vivo experiment

SLN-P80 were labeled with cardiogreen fluorophore to allow their visualization in living organisms, and the in vivo biodistribution was evaluated with Fluorescent Luminescent Imaging (FLI). Mice, athymic male (n = 8) (Harlan Laboratories), about 4-5 weeks old and 25 g in weight were divided into two groups, the first one (n=4) was administered with an intraperitoneal (IP) injection with 500 µl of fluorescent SLN-P80, 50mg/ml in lipid phase, the second one (n=4) was administered with an IP injection with 500 µl of buffer solution. During injection and image acquisition, mice were kept under gas anesthesia (2% of isoflurane and 1 l/min of oxygen). Optical images were acquired with IVIS Spectrum (Perkin Elmer) in fluorescent modality. After 4 h from systemic administration, in vivo images show an SLN-P80 specifically accumulation in the superior abdominal area correspondent to the liver one (Figure 1a). After the last acquisition of the in vivo protocol, mice were sacrificed through an anesthetic overdose and they were perfused with a solutions with 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The organs of interest were excised and re-acquired with the same experimental protocol and it was evident the fluorescence emission from the liver (Figure 1b).

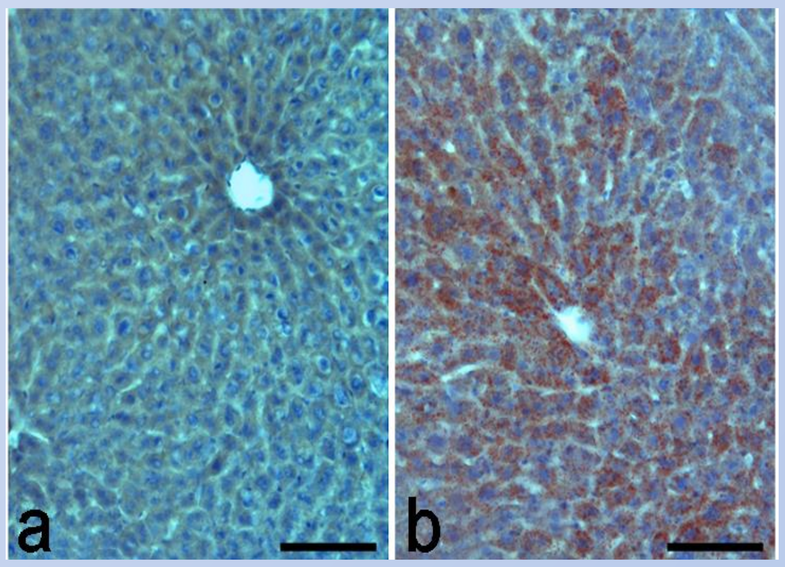

Figure 2. LM: control (a) and SLN-P80-treated (b) liver; note the large amounts of lipid droplets (red oil) in b. Bar, 100 µm.

Ex vivo experiment

The excised liver was analyzed at light microscopy (LM) (Figure 2) to evaluate SLN-P80 specific localization after 4h post systemic administration in vivo. The excised liver post perfusion of the mouse was further fixed by immersion in liquid nitrogen, and stored at -80°C until use. Cryostat sections of a 7-µm thickness were stained with Oil Red O solution (Bio Optica), as reported in the datasheet, and Mayer's hematoxylin solution (Sigma).
LM revealed that no histological alteration and liver injuries were induced by SLN-P80 systemic administration. There are not necrotic areas in the treated liver and the hepatic morphology was conserved.
However, a marked increase in lipid content (so as it is showed by the Oil Red staining in figure 2b) was found in hepatocytes, especially close to the centrilobular venula, according to the hexagonal lobule concept.
To clarify this phenomenon, we investigated in vitro the uptake and intracellular fate of SLN-P80 in a murine cell line.

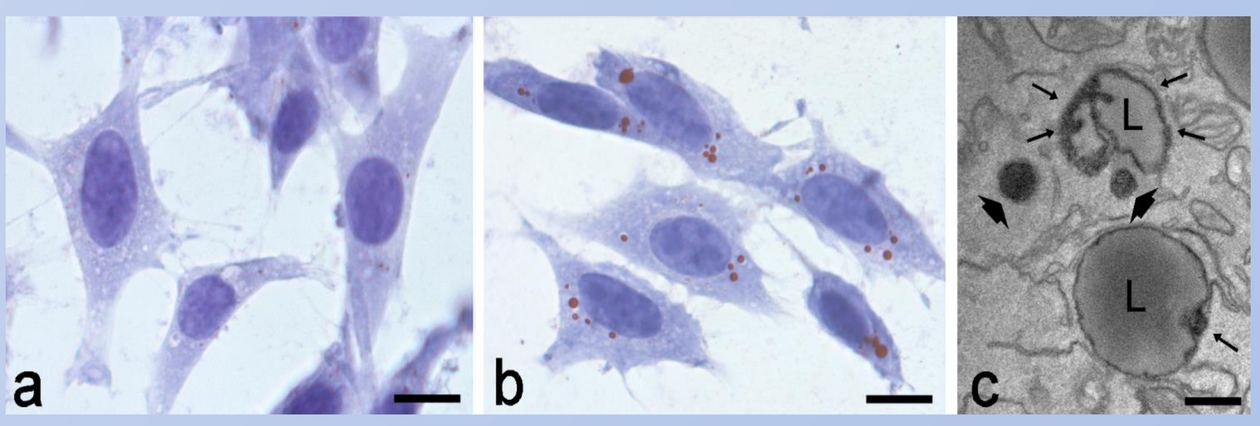

Figure 3. LM: 3T3 cells incubated with 0.5 mg/ml of sterilized SLN-P80 for 1h, 4h and 24h (b) contain more lipid droplets (red oil) than controls (a). Bars, 20 µm. TEM (c): SLN-P80 (arrows) occur near to lipid droplets (L) containing electron dense material (thin arrows). Bar, 200 nm.

In vitro experiment

3T3L1 cell was used as the in vitro model. 3T3-L1 cells were plated at a density of 5000 cells on glass coverslips at 37°C. After 24h, the medium was replaced with fresh medium containing 0.5 mg/ml of sterilized SLN-P80. After 1h, 4h and 24h of incubation, the cells were fixed with 4% buffered formalin for 13 min, rinsed with PBS and stained with Oil Red O (Bio Optica) and Mayer's hematoxylin solution (Sigma). It could be observed an increase of the lipid content of the cells (Figure 3b) than control cells (Figure 3 a). For TEM, samples were fixed with glutaraldehyde 2,5% and 2% paraformaldehyde, post-fixed in OsO_4, and embedded in Epon. The ultra-thin sections were stained with lead citrate and observed using a Morgagni 268D electron microscope (FEI Company). At TEM scarce SLN-P80 were observed inside the cells: they occurred free in the cytoplasm, often in close proximity to lipid droplets showing a peripheral electron dense material (Figure 3c). This suggests that SLN-P80 enter the cells by fusion with the plasma membrane, undergo rapid degradation and their components migrate, probably due to chemical affinity, into the lipid deposits.

Conclusion: Our results clearly demonstrate the high biocompatibility of SLN-P80: in the living organism they physiologically accumulate in the liver, which does not undergo histological damage in spite of the high dose of administered nanoparticles. This biocompatibility is likely due to both the high biodegradability and the chemical composition of SLN-P80. In fact, the degradation products of nanoparticles are stored into the hepatocytes (probably in their lipid droplets) similarly to lipid metabolites.

References
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