In vivo and in vitro biodistribution of solid lipid nanoparticles

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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).
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) after intraperitoneal (i.p.) administration in a mice model. After the in vivo acquisition, mice were perfused and the excised organs (liver, kidney, spleen, brain, lung) were acquired to confirm SLN-P80 accumulation in specific districts (3). Organ tissues were also analyzed at light microscopy (LM) to evaluate SLN-P80 localization. After 4 h from systemic administration, SLN-P80 specifically accumulated in liver (Fig. 1a). LM revealed that no histological alteration is induced by SLN-P80 administration; however, a marked increase in lipid content (red oil staining) was found in hepatocytes, especially close to the centrilobular venula (Fig. 1b,c). To clarify this phenomenon, we investigated in vitro the uptake and intracellular fate of SLN-P80 in a murine cell line. 3T3 cells were incubated with SLN-P80 for 1, 4 and 24h, and then processed for both LM (red oil staining) and transmission electron microscopy (TEM). SLN-P80 administration induced accumulation of lipid droplets already after 1 h (Fig. 2a,b). 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 (Fig. 2c). 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.

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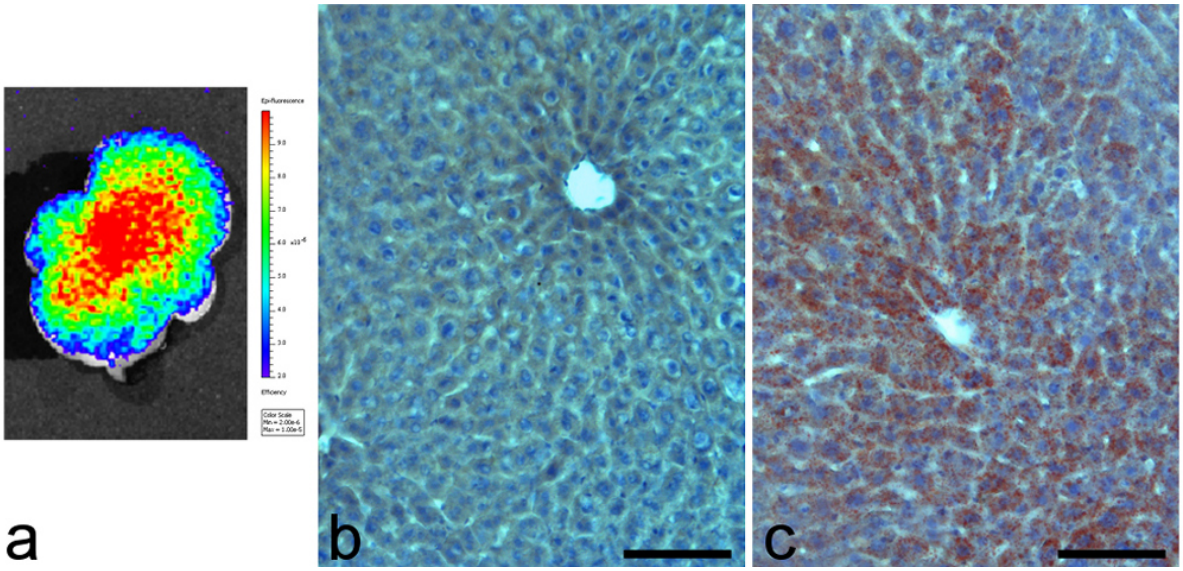

Figure 1. FLI : liver explanted from a mouse 4h after perfusion with SLN-P80 (a). LM: control (b) and SLN-P80-treated (c) liver; note the large amounts of lipid droplets (red oil) in c. Bar, 100 µm.

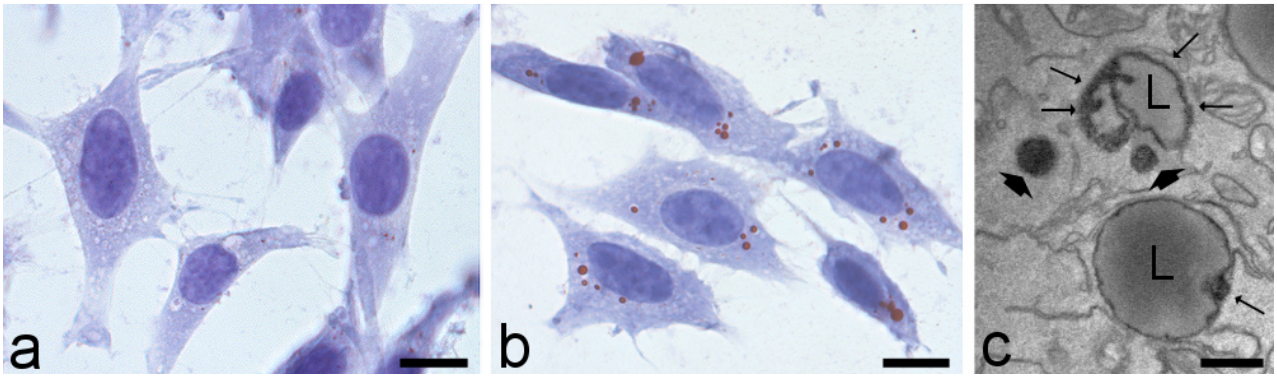

Figure 2. LM: 3T3 cells incubated with SLN-P80 for 1h (b) contain more lipid droplets (red oil) than controls (a). Bars, 20 µm. TEM: SLN-P80 (arrows) occur near to lipid droplets (L) containing electron dense material (thin arrows). Bar, 200 nm.