Abstract: Super Stealth immunoliposomes as a strategy to overcome liposome-induced liver toxicity

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Background and Aims: Immunoliposomes (ILs) are nano-delivery systems functionalized with monoclonal antibodies or antibody fragments, with the double aim of ameliorating the pharmacokinetics and tolerability of incapsulated drugs and permitting targeted therapy. Binding poly-ethylene-glycol (PEG) chains to their surface, thus obtaining stealth ILs, delays their elimination, which is mainly due to the clearance operated by the reticuloendothelial system (RES). Recently, to further improve their biopharmaceutical and pharmacokinetic features, the so called "super stealth liposomes" have been proposed, by adding mPEG-dendron-phospholipids. However, these nano-sized materials can accumulate in the liver and cause hepatic toxicity. On the basis of these considerations, the aim of this study was to evaluate, at the histological and molecular level, the *in vivo* liver toxicity of two formulations, one of stealth (SIL) and one of new super-stealth IL ($SSIL_2$), both loaded with doxorubicin, in Sprague-Dawley rats.

Method: A dose of 2.5 mg/kg of doxorubicin-loaded immunoliposomes (SIL and $SSIL_2$) was administered via caudal vein to Sprague-Dawley female rats (n=3 per group) and vehicle-administered rats were used as controls. Rats were sacrificed 48 hours after the treatment. Hepatic toxicity of the formulations was assessed by: 1) standard histological analysis performed on 5-μm sections of liver tissues stained with H&E; 2) mRNA hepatic expression of IL-1β, IL-6 and TNF-α; 3) reactive oxygen species (ROS) concentration in liver tissues. The results were compared by one-way ANOVA followed by Dunnett's *post-hoc* test. $p<0.05$ was considered statistically significant.

Results: Rats treated with SIL showed hepatic histological alterations, i.e. numerous granulomatous lesions, sometimes associated with apoptotic bodies, whereas in rats treated with $SSIL_2$ only few isolated granulomas could be observed in the otherwise healthy livers. The expression of both IL-1β and TNFα was significantly increased only in in SIL-treated rats ($p <0.001$ vs controls) and did not change in $SSIL_2$ – treated rats with respect to controls. Accordingly, the concentration of hepatic ROS increased significantly in SIL-treated rats ($p<0.001$ vs controls) and was comparable to that of controls in $SSIL_2$ – treated rats.

Conclusion: SILs are able to induce dramatic alterations of the hepatic parenchyma, probably due to their preferential deposition in hepatic tissue, which is particularly rich in RES cells. Conversely, $SSIL_2$ caused only limited histological liver alterations. Therefore, $SSIL_2$s, besides their pharmacokinetic advantages, permit to overcome the hepatic toxicity caused by SIL administration, thus representing a smart strategy to improve the tolerability of cancer therapy.

Figure: