Safety of inhalable biodegradable nanoparticles

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Nanoparticles (NPs) offer great potentialities for pulmonary drug delivery particularly for targeting alveolar macrophages. Addressing nanoparticles to macrophages in was achieved using poly(lactide-co-glycolide) (PLGA) nanoparticles. These NPs were modified in a bacteriomimetic manner being covered by mannose and surfactant protein A (SP-A) mimicking the pathway by which *Mycobacterium tuberculosis* enter alveolar macrophages. However, the very same properties that make biodegradable NPs exciting drug carriers might induce harmful effects as they interact with many cells. Using *in vitro* and *in vivo* models, we have tried to correlate the potential toxicity of PLGA NPs to their cellular bioavailability or biodistribution as well as their physicochemical properties. For this purpose, three types of surface-modified NPs were designed: positively and negatively charged as well as neutral. NPs were prepared using PLGA. Positively and negatively charged as well as neutral PLGA NPs were obtained by coating their surface with chitosan (CS), poloxamer (PF68) or poly(vinyl alcohol) (PVA), respectively.

We have first used an *in vitro* model of Calu-3 cell to mimic the bronchial epithelial barrier. The role of NP surface chemistry and charge on the epithelial resistance and mucus turnover was investigated. MUC5AC was used as a marker of mucus production. It was shown that the interaction with mucin reduced the penetration of CS- and PVA-coated NPs while the hydrophilic PF68-coated NPs were able to diffuse across the mucus barrier leading to a higher intracellular accumulation. NPs did not interfere with the formation and maintenance of tight junctions, with the exception of CS-coated NPs which caused a transient but reversible decrease of the trans-epithelial electrical resistance. NPs did not increase the MUC5AC mRNA expression or the protein levels regardless of their surface properties. Moreover, no inflammation was observed as evaluated by measurement of proinflammatory cytokines.

A co-culture model of THP-1/A549 cells was then used to evaluate the toxicity of biodegradable NPs. This model was shown to be relevant for *in vitro* pulmonary nanotoxicology studies. It was possible to detect a mild inflammatory response to PLGA NPs stabilized by three different hydrophilic polymers PVA, CS and PF68, but very limited compared to well-known inflammatory compounds. *In vivo* in mice the administration of biodegradable NPs did not induce an inflammation process as opposed to non-biodegradable NPs for which all parameters measured clearly evidenced acute toxicity after intratracheal administration.

In conclusion, we have shown that it was possible to deliver PLGA NPs into microparticulate forms and moreover to functionalize these NPs for better targeting of macrophages. In addition, whatever is the surface modification, we found out little adverse effects of these particles *in vitro* and *in vivo*.