Inhalated drug delivery systems to target alveolar macrophages for tuberculosis therapy: design of safe SLM loaded with rifampicin

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One-third of the world's population is infected with tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis* which survives and replicates within human macrophages. TB is characterized by a long chronic stage of infection and progressive pathology that mainly compromises (90% of cases) the respiratory system (World Health Organization, 2012). The current recommended TB chemotherapy includes: isoniazid, rifampicin, ethambutol and pyrazinamide. However, patient's noncompliance, several side effects and multi-drug resistant TB infections, often make ineffective the therapy. The present research aimed to improve the effectiveness of the treatment by a non conventional therapy and using the respiratory tract as a novel administration route for rifampicin. This approach involved the design of solid lipid microparticles (SLM) as drug delivery system (DDS) showing benefits in terms of biocompatibility, easy of preparation and scale-up, low cost of materials and procedures (Muttil et al., 2009). The study, in its first phase of implementation, dealt with the formula optimization in order to provide SLM characterized by physicochemical properties proper for a delivery by a dry powder inhaler (DPI) device and for the targeting to alveolar macrophages. SLM (~1 μm) loaded with rifampicin were composed of stearic acid and sodium taurocholate as the lipid matrix and the surfactant, respectively. These materials were selected for biocompatibility and capacity to produce anionic particles that promote uptake by the alveolar macrophages (Kelly et al., 2011). The preparation process involved O/W emulsification and lipid phase solidification technique by means of sonication followed by freeze-drying to generate a final respirable powder. SLM showed a nearly round shape, smooth surface, homogeneous dimensional distribution with mean size of 1 μm, negative Z-potential value and low bulk and tap density as the evidence of high porosity. The aerodynamic diameter that represents the breathability of the powder was found proper (~0,5 μm) to transport the microparticles until alveolar epithelium, i.e. the therapy target. Drug loading level was about 4% (w/w) with an encapsulation efficiency of about 80%. Test: SLM internalization by macrophages was evaluated on the murine macrophages cell line J774 cultured in DMEM medium and nile red was chosen as selective fluorescent stain. Three SLM samples were assayed for their ability to be taken up by J774 cells: unloaded SLM, unloaded SLM nile red labelled, rifampicin loaded SLM nile red labelled. Results: The negligible *in vitro* release of rifampicin from SLM indicated the capacity of the matrix to firmly entrap the drug preventing its spreading over the lung fluid before the occurring of SLM uptake by alveolar macrophages. A microbiological assay performed with rifampicin loaded SLM on *B. subtilis* strain demonstrated that the drug antimicrobial activity is preserved in SLM just indicating that materials and procedures do not interfere with the drug biological activity. SLM were found non cytotoxic (MTT test) and able to be taken up by cell cytoplasm as demonstrated by flow cytometry and confocal laser microscopy. Conclusions: The microparticulate DDS designed and characterized in the present study could be considered promising in a perspective of an efficacious TB inhaled therapy by means of DPI inhaler. The next step will consider other important factors as the evaluation of the emitted dose by the device, the respirability of the sample by a cascade impactor and by no means negligible, the activity of drug loaded SLM on *M. tuberculosis* strains.

World Health Organization, *Global Tuberculosis Report 2012*.
Muttil et al. (2009). *Pharm Res.* 26, 2401-2416.
Kelly et al. (2011). *J Drug Del.* 2011, 1-11.