1. INTRODUCTION, AIMS AND OBJECTIVES

1.1 Introduction

It has been for a long time one of the great challenges for pharmacists to deliver poorly soluble drugs efficiently to the target tissue. The contribution of this thesis is the better understanding of the strategies in order to prepare formulations based on drug nanocrystals for lung delivery with state of the art application devices. In this work the naphthoquinone buparvaquone has been selected as model drug for its poor water solubility. Additionally, the goal was to prove the efficacy of buparvaquone nanocrystals, prepared in so called nanosuspensions, in the treatment of *Pneumocystis* pneumonia (PcP) *in vitro* and *in vivo*.

Poorly water soluble drugs are specially challenging, as they cannot achieve dissolution and therefore they have a very difficult pass through the dissolving fluid to contact the absorbing mucosa and to be absorbed. If the dissolution process of the drug molecule is slow, due to the physicochemical properties of the drug molecules or formulation factors, then dissolution may be the rate-limiting step in absorption and will influence drug bioavailability. This is the case of class II drugs, e.g. buparvaquone (according to the Biopharmaceutics drug Classification System BCS). For this specific kind of drugs, micronization (Steckel, Thies et al. 1997; Rasenack, Steckel et al. 2004), nanonization (Ostrander, Bosch et al. 1999; Jacobs and Müller 2002), complexation (e.g. cyclodextrins) (Skiba, Bounoure et al. 2005), preparation of liposomes (Waldrep, Arppe et al. 1997; Arppe, Vidgren et al. 1998) and amorphous solid dispersions (van Drooge, Hinrichs et al. 2005) has been proposed to increase rate of dissolution and drug bioavailability of drugs targeting the lung for local or systemic drug absorption, using the pulmonary route of administration by employing devices such as nebulizers, pressurized metered dose inhalers and dry powder inhalers.

The lungs provide an excellent surface for absorption; this is probably its most attractive characteristic that makes the lung the ideal object for studying drugs targeting systemic and not only respiratory diseases. For both kinds of applications, the most important factor for success in the formulation of drugs for pulmonary administration is the aerodynamic diameter of the particles to be delivered. Either for inhaled powders or for droplet aerosols, the aerodynamic behaviour of the drug formulated to be delivered to the lung (aerodynamic size distribution, density, shape, etc.) will define the site of deposition. Particles within the range of 3-5 μm preferentially deposit in the upper airways, while particles from 2-3 μm have a higher

probability to reach the deep lung. In the case of devices producing aerosol droplets such as nebulizers, the size distribution of micronized drugs is typically not capable of ensuring that the droplets produced by the aerosolization of aqueous formulations will contain drug. In this thesis, nanocrystals are used as a very suitable approach to solve this problem. The production of nanocrystals by means of the high pressure homogenisation technique allows the reduction of the particles average size distribution within the nanometer range and with 99 % of the drug nanocrystals being smaller than 1-1.5 μm. This will ensure the incursion of drug in small droplets produced by nebulizers designed to deposit the formulation in the peripheral region of the lung (2-3 μm droplet size). Furthermore, the production of nanosuspensions offers the possibility to incorporate a high concentration of drug in a relatively low volume of fluid, which is an important advantage in the delivery of antimicrobials. Nanosuspensions containing higher concentrations of dispersed drug such as 50-100 mg/mL produced by high pressure homogenisation will present low viscosity, facilitating their nebulization, reducing the nebulization times and hence increasing patient compliance. The characterization of wet aerosols produced via nebulization of nanosuspensions including the *in vitro* determination of the efficiency of the delivery system is extensively investigated in this thesis. Another important application investigated in this thesis is the formulation of nanocrystals as suitable powders for inhalation. This can be derived from the encapsulation of nanocrystals in a polymer or in sugar carriers using spray-drying or spray-freeze drying processes, which yield spherical particles with an accelerated rate of dissolution and a high stability, as the solid state of the drug is crystalline.

As a proof of concept of the use of nanocrystals administered in aerosol droplets, the administration of buparvaquone nanocrystals by means of a nebulizer device in the therapy of Pneumocystis pneumonia (PcP) was developed in this work. PcP is a potentially fatal opportunistic infection in immunocompromised patients. Prior to the use of highly active antiretroviral therapy (HAART), PcP affected nearly 80 % of all AIDS patients at some point of their course (Phair, Munoz et al. 1990; Stringer, Beard et al. 2002). Approximately 20-50% of patients have significant adverse reactions to the current standard therapy, trimethoprim-sulfamethoxazole (TMP-SMX), which results in those patients being switched to potentially less effective medications. In addition, PcP resistance to TMP-SMX, and subsequent treatment failure, has been reported (Saah, Hoover et al. 1995). PcP is also of clinical importance in people

immunocompromised for reasons other than HIV, such as organ transplantation or chemotherapy for malignant diseases (Hughes 1978) or those who are unaware of their HIV status. In addition, *Pneumocystis* infection has been documented recently in persons who are mildly immunocompromised, including those with chronic lung disease (Contini, Villa et al. 1998). There is until now no adequate cultivation method that provides sufficiently purified organisms suitable for many types of experiments. Therefore, the testing of new therapies against the infection as the one proposed here with the drug buparvaquone, can only be obtained by testing in small rodent infection models (i.e. rats, mice).

1.2 Aims and objectives

1.2.1 Formulation development

- Formulate buparvaquone nanosuspensions suitable for pulmonary application using regulatory accepted surfactants.
- Investigate the chemical (drug content) and physical (nanoparticles size distribution) stability of the nanosuspensions formulated.
- Investigate the improvement in dissolution rate and saturation solubility of the buparvaquone nanocrystals prepared.

1.2.2 *In vitro* application of buparvaquone formulations

- Perform the *in vitro* characterization of the aerosol emitted from jet and ultrasonic nebulization technologies. Investigate the influence of physicochemical characteristics of different nanosuspensions such as viscosity, surface tension, density, drug concentration, on the aerosol droplet size produced using both types of nebulizers. Determine the degree of nanocrystals aggregation as a result of the nebulization technology and formulation properties.
- Characterize the percentage of dose emitted by a jet nebulizer capable to produce especially small droplet sizes (Pari LC Star) when nebulizing nanosuspensions with different physicochemical properties. To determine the respirable dose of the aerosol emitted (aerosol droplets < 3 µm).

1.2.3 Buparvaquone solid dispersions

- Prepare solid dispersions by spray-drying and spray-freeze-drying containing drug nanocrystals using the polymer PVP and the oligosaccharide inulin as carriers.
- Prepare solid dispersions solubilizing the drug in ter-butyl alcohol instead of nanonization, and the further incorporation of such solutions into solid particles by spray-drying and spray-freeze-drying.
- Characterize the products to identify the solid state of the drug and the improvement in dissolution obtained using these techniques.

1.2.4 *In vivo* application of buparvaquone nanosuspensions

- Use *Pneumocystis* pneumonia infected mice to test buparvaquone nanocrystals administered through aerosol droplets as a treatment.
- Develop the infection in immunocompromised mice.
- Characterize the aerosol produced by the whole-body inhalation chamber used for the therapy.
- Evaluate the infection status in all group of animals (treated and not treated) using specific methodology for the quantification of the infection burden and the quantification of drug in different organs.

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