DEVELOPMENT AND PHARMACOKINETIC EVALUATION OF NANOPARTICLES LOADED WITH QUININE/DOXYCYCLINE

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Introduction: Malaria is one of the most devastating tropical diseases caused by intracellular protozoan parasites of the genus *Plasmodium*. More than 3 billion people live in malarial endemic regions. Five species of *Plasmodium* (*falciparum, vivax, ovale, malariae* and *knowlesi*) cause disease in humans and infection with *P. falciparum*, the most deadly of these parasites, results in more than 1 million deaths annually. The development of resistance to traditional antimalarial drugs lead to the use of drug combinations such as quinine (QN)/doxycycline (DOX).[1,2]
Objective: The objectives of this part of the project were to develop, characterize and to evaluate the pharmacokinetics (PK) of nanoparticles loaded with QN/DOX aiming to increase drugs erythrocyte penetration leading to decreased drug doses and quinine toxicity.
Materials and Methods: The developed solid lipid nanoparticle formulations (SLN) were prepared as follows: the oil phase was composed of cetyl palmitate, QN and lipoid 40 and the aqueous phase were composed of water, DOX and Tween 80®. When the oil phase reached 75 °C and water phase 80 °C they were mixed and kept under agitation in ultraturrax for 10 min. The formulation was recirculated three times in high-pressure homogenizer. The formulations were evaluated for 21 days for the following physico-chemical parameters: drug content, pH, diameter, zeta potential and polydispersity index. Drug content was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) method previously validated[3]. Moreover, a bioanalytical LC-MS/MS method was validated for the determination of QN and DOX in rat plasma. The adequacy of the bioanalytical method for PK determinations was investigated in a pilot study after QN and DOX (free base or nanoencapsulated form) dosing to Wistar rats (n = 5/group) (Approved by UFRGS Ethics in Research Committee). The groups received a single 25 mg/kg of QN and 2.5 mg/kg of DOX i.v. *bolus* or orally. Plasma samples were harvested at pre-determined time points up to 24 h. PK parameters were determined by non-compartmental approach.
Results and Discussion: In the optimized formulation of SLN we used cetyl palmitate as lipid phase and a mixture of lipoid 40 and Tween 80® as emulsifier. It is known that the use of two emulsifiers, respectively of lipophilic and hydrophilic nature, yields a better stabilization of the disperse system. In the physico-chemical evaluation, the obtained results of particle size (153 nm), polydispersity index (0.176), negative zeta potential (−37 mV) and drug content (95.2% for QN and 96.8% for DOX) demonstrated that the formulations were stable for 21 days. The formulation was considered adequate to be administered to animals viewing to determine the pharmacokinetics of the entrapped drugs as well as to investigate the changes in effect due to encapsulation. The validated bioanalytical method was linear in the concentration range of 5-5000 ng/mL for both compounds. The validation parameters (specificity, recovery, linearity, precision, accuracy, and stability) gave results within the acceptable range. The bioanalytical method proved to be suitable for PK study because both drug profiles were properly characterized, leading to extrapolated areas under the curves smaller than 4.0% for both drugs.
Conclusions: In conclusion, up to now the results showed that it is possible to produce QN/DOX loaded nanoparticles with adequate physico-chemical and stability characteristics. Additionally, a bioanalytical method for simultaneous quantitation of QN and DOX in rat plasma was developed and validated. The method was successfully applied to a pharmacokinetic study in rat. The perspectives of this part of the project are to investigate the pharmacokinetics of the entrapped drugs in rodents and to determine whether the SLN developed are capable of increasing QN/DOX infected erythrocyte penetration.

References:
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