

DEVELOPMENT OF A SENSITIVE ANALYTICAL METHOD FOR SIMULTANEOUS QUANTIFICATION OF RAPAMYCIN AND CYCLOSPORINE IN MOUSE WHOLE BLOOD AND DETERMINATION OF THEIR PHARMACOKINETIC INTERACTIONS WITH INTRAPERITONEAL DELIVERY

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Background: Rapamycin (RAP, sirolimus) is used as an immunosuppressant to minimize host rejection in organ transplantation. It has also shown promise in pre-clinical and recently clinical studies as an antineoplastic agent for preventing malignancy after transplantation [1]. Ongoing studies in our laboratories show promise for its use as a single agent or in combination with cyclosporine A (CSA) for reducing occurrence of skin cancer in a laboratory mouse model. To further understand these favorable effects, a quantitative analytical method was developed to simultaneously measure whole blood concentrations of both compounds. Studies are underway to determine the pharmacokinetics of rapamycin and its potential drug-drug interactions with cyclosporine A, both substrates for CYP3A enzymes and P-glycoprotein. **Objectives:** The aims of our study are to 1) develop and validate an analytical method for simultaneous quantitation of RAP and CSA in mouse whole blood, 2) determine relevant pharmacokinetic parameters for RAP, and 3) identify potential pharmacokinetic interactions of the two compounds. **Methods:** Due to their primary distribution in red blood cells, a method for extraction of both compounds from whole blood was developed. An LC/MS/MS assay was developed with reversed phase separation of reconstituted extracts on a diphenyl column and acidified (0.1% acetic acid) water and acetonitrile mobile phases. Ionization of RAP, CSA, and the internal standard, ascomycin, was achieved via atmospheric pressure chemical ionization (APCI) with single reaction monitoring for each compound. Female hairless SKH1 mice, 8 – 12 weeks old, were dosed with a single i.p. injection of rapamycin (2 mg/kg) alone or in combination with cyclosporine A (10 mg/kg). Whole blood was collected via cardiac puncture at various times ranging from 3 min. to 55 hours and stored at -80°C until extraction and analysis. Blood concentration versus time data was analyzed in WinNonlin. **Results:** The lower limit of quantitation of the analytical method for both rapamycin and cyclosporine was 1 nM with a linear range up to 10 uM (rapamycin) or 30 uM (cyclosporine A). Results from a pilot study indicated a two-compartment model may best describe the kinetics for both compounds with elimination half lives of ~13 and ~11 hours for RAP and CSA, respectively. Analysis of the full study is underway.

1. Buell, JF, et al. Malignancy after transplantation. *Transplantation*, 2005. **80**(2 Suppl): S254-64.

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