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Cyclosporine G Analysis and Monitoring by High-Pressure Liquid Chromatography

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CYCLOSPORINE G, or Nva²-cyclosporine (CsG), is a member of a class of compounds produced by the fungus *Tolypocladium inflatum* GAMS that have marked immunologic activity. Cyclosporine (CsA) is the most extensively studied compound of the group and has been very successful in clinical use as an immunosuppressant in organ transplantation. The major problem with the clinical use of CsA is its propensity to produce nephrotoxicity in patients at a wide range of doses. CsG is very similar structurally to CsA and has been reported to lack nephrotoxicity in a rat model.¹

CsG has been shown to be equally potent as CsA in preventing rejection of transplanted livers in the canine model.² Any comparative study of CsA and CsG should also assess potential pharmacokinetic differences in the two compounds. Previous studies have shown that the distribution of cyclosporine in blood is highly dependent on the temperature. A knowledge of the temperature-dependent distribution of CsG in blood is therefore essential for designing a pharmacokinetic study. The following study was designed to support a comparative study of CsA and CsG in canine liver transplantation. Our objectives were to (1) establish an assay for CsG; (2) investigate the effect of temperature on blood and plasma concentrations of CsG; and (3) assay canine samples over a course of therapy with CsG.

MATERIALS AND METHODS

CsG Assay

CsG was assayed using high-pressure liquid chromatography (HPLC). CsA was used in the internal standard. Blood and plasma samples were extracted by a previously described procedure.³ The final residue was dissolved in the mobile phase (200 μ l) and injected (150 μ l) onto the HPLC column.

A 5- μ , 15-cm, C₁₈ column (Supelco, Bellefonte, PA) heated to 70 °C was used to separate compounds of interest. The mobile phase consisted of 68:32 acetonitrile:water pumped at a rate of 1.5 mL/min. CsA and

CsG were quantitated by their absorbance at 214 nm (Waters Model 441, Milford, MA). Under these conditions, the retention time for CsA and CsG were 7.1 and 9.7 minutes, respectively.

Recovery, Reproducibility, and Accuracy

CsG was spiked into blank blood or plasma in the concentrations of 50, 250, 500, 1,000, and 2,000 ng/mL to assess the recovery of the drug using the liquid extraction. The internal standard (CsA, 250 ng) was added at the end of the extraction, and a standard curve of the peak height ratios (CsG/CsA) v CsG concentration was established. Percentage of recovery was calculated as the slope of the extracted drug standard curve divided by the slope of the unextracted drug standard curve (methanolic solution of CsG) times 100. The accuracy of the assay was determined by spiking whole blood with CsG (1,240 ng/mL) and performing eight repetitions of the drug extraction and assay. Assay reproducibility was assessed at 250 and 500 ng/mL by assaying six sets of spiked whole blood samples.

Stability

CsG stability in whole blood was determined at room temperature and at 46 °C for a period of ten days.

Blood-Plasma Ratio

The effect of temperature on the blood-plasma ratio of CsG was determined at 5, 25, and 37 °C. Fresh heparinized blood was spiked with 1,240 ng/mL CsG and was maintained at the appropriate temperature for two hours. The blood was then spun in a thermostatically controlled centrifuge to obtain the plasma, which was assayed along with the whole blood.

Canine Studies

Female beagle and mongrel dogs underwent orthotopic liver transplantation and were randomly selected to

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receive either CsA or CsG. The dogs received either drug in an oral olive oil solution in the dose of 20 mg/kg for the first 30 days following transplantation, after which the dose was reduced to 15 mg/kg body weight. Blood was obtained every third day in the morning for HPLC analysis of CsG or CsA.

RESULTS

Extracts of blank blood and plasma indicated the lack of interference of any endogenous materials with CsG or CsA. The minimum detectable concentration of CsG was 25 ng/mL using 1 mL of blood or plasma. The HPLC assay for CsG was linear over 50 to 2,000 ng/mL as demonstrated by consistent correlation coefficients of greater than 0.998. The liquid-liquid extraction procedure used in the present assay yielded recovery values of 90% for CsG in both blood and plasma. In the accuracy study, the whole blood was spiked with 1,240 ng/mL of CsG and was assayed as having 1,156 ng/mL on eight repetitions, representing a percentage of error of 6.8%. The coefficient of variation of this assay was 4.0% at 250 ng/mL and 2.5% at 500 ng/mL for CsG in whole blood. Assays performed on spiked whole blood maintained at room temperature or at 46 °C for up to ten days indicated no loss of CsG from the samples.

The distribution of CsG in blood is temperature dependent. The blood-plasma ratio (B/P) is 1.45, 1.2, and 0.9 at 5 °C, 26 °C, and 37 °C, respectively.

The trough concentrations of CsG in an individual dog over the course of therapy are shown in Fig 1. The dose reduction on day 30 did not result in a marked decrease in trough CsG concentrations. The morning predose whole blood CsG concentrations generally increased over the first 20 to 30 days of therapy. The concentration of CsG was significantly higher than CsA concentrations at days 36, 57, 63, 66, 69, 81, 84, and 87 of therapy ($P < 0.05$ by t test). The average trough concentration of CsG (409 ng/mL) was higher than that of CsA (236 ng/mL). The dogs in both groups had a significantly longer survival time ($P < 0.05$) when their trough blood concentration was greater than 200 ng/mL.

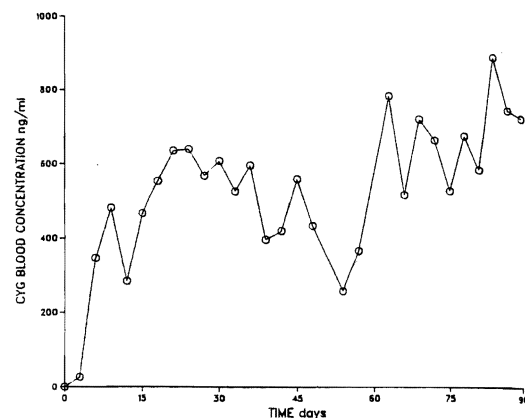


Fig 1. CsG blood concentration v time in a dog who was given 20 mg/kg/day CsG on days 0 to 30 and 15 mg/kg/day on days 30 to 90 following orthotopic liver transplantation.

DISCUSSION

CsG is a promising immunosuppressant for use in organ transplantation. CsG has been shown to be as effective as CsA in preventing organ rejection in rodent transplant models¹ and in the canine studies for which the HPLC CsG assay was developed.² Nephrotoxicity has not appeared in toxicologic rat studies with CsG¹ and was not a problem in the canine study even though CsG blood concentrations were frequently high. Decreased nephrotoxicity would provide CsG with a significant advantage over CsA while other biopharmaceutical problems, such as intersubject variability in pharmacokinetics, would most likely remain.

The HPLC assay described for CsG demonstrates the flexibility of HPLC for monitoring immunosuppressive compounds of cyclosporine family. Only minor modifications of the HPLC assay of CsA are necessary to provide accurate and reproducible blood concentration of CsG. A number of compounds of the cyclosporine family were tried as internal standards; optimal resolution was obtained with CsA as the internal standard.

Preliminary studies with solid phase extraction techniques yielded poor and variable recovery of CsG from blood. The liquid-liquid extraction procedure used in this assay consistently provided 90% recovery of CsG from

whole blood and plasma. Plasma CsG can be extracted using solid-phase extraction procedures. However, less than 70% of the drug is extracted by this method.

Similar to what has been observed with CsA, the B/P of CsG is a function of temperature. As the temperature increases, less and less cyclosporine partitions into erythrocytes. In theory, this may be due to increased uptake of CsG by proteins in the plasma or decreased uptake by erythrocytes at higher temperatures. Preliminary evidence suggests that erythrocyte uptake is independent of temperature. Irrespective of the mechanism involved, plasma samples should be separated at 37 °C in order to obtain meaningful pharmacokinetic parameters for CsG.

Following oral administration, a wide range of trough concentrations of CsG were observed in dogs even though a consistent dose per kilogram of body weight was being administered. While this variability was predicted from observations with CsA, the higher blood concentrations of CsG than CsA were unexpected. While increased absorption could account for higher CsG blood concentrations, its poor water solubility (similar to CsA)

makes improved absorption of CsG over CsA unlikely. Altered distribution of CsG, due to variable binding to red cells or to plasma lipoproteins, or differences in hepatic drug clearance, are equally likely to have produced higher CsG than CsA blood concentrations.

SUMMARY

HPLC provides an accurate and reproducible means of measuring CsG in blood or plasma using CsA as an internal standard. The blood-plasma ratio for CsG varies with temperature of separation so that whole blood monitoring is preferred. CsG blood concentrations were higher than CsA blood concentrations when equal doses of both drugs were administered to dogs over a 90-day period. Pharmacokinetic differences are therefore predicted between CsA and CsG.

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