Research Article

Saturable Binding of Cyclosporin A to Erythrocytes: Estimation of Binding Parameters in Renal Transplant Patients and Implications for Bioavailability Assessment

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Cyclosporin (CyA) exhibits saturable binding to erythrocytes. A one-site binding model was fitted to data from renal transplant patients receiving CyA therapy. The average maximum binding capacity is 2500 μg CyA/liter of packed erythrocytes; the unbound CyA concentration associated with 50% saturation of the binding site is 67 μg/liter. Analysis indicates that whole-blood CyA measurement to monitor drug therapy should be viewed cautiously, particularly when the hematocrit varies considerably. The error in estimating absolute bioavailability at steady state from whole-blood measurements, resulting as a consequence of the saturable binding, has been explored. Although extrapolation to the therapeutic situation, which involves transient drug administration, is difficult, errors of up to 25% are anticipated. When an accurate estimate of bioavailability is required, analysis based on plasma data is proposed. For bioequivalence testing, both blood and plasma CyA data are equally acceptable.

KEY WORDS: cyclosporin; saturable binding; erythrocyte; bioavailability; therapeutic drug monitoring.

INTRODUCTION

Cyclosporin A (CyA) is a third-generation immunosuppressant dosed chronically to transplant patients (1). Owing to its narrow therapeutic index and relatively soft indices of efficacy and toxicity, both blood and plasma concentration monitoring of CyA has become commonplace. However, the quantitative distribution of CyA within blood is poorly defined.

Cyclosporin binds to both erythrocytes and plasma proteins, with the ratio of the concentration of CyA in blood to that in plasma (Cp/Cp) being concentration dependent (2). In man, the binding of CyA to plasma proteins is independent of concentration (3,4), which implies that any concentration dependence of Cp/Cp is due to saturable binding of the drug to erythrocytes. Saturable erythrocyte binding of CyA has also been observed in rabbit blood in vitro and a model, based on a single binding site, has been applied to these data (5).

In this paper we present data to confirm saturable erythrocyte binding in renal transplant patients undergoing CyA therapy and estimate the parameters for the single-binding site model. The implications of saturable erythrocyte binding in the assessment of bioavailability of CyA from plasma and whole-blood data are also examined by use of the model.

MATERIALS AND METHODS

Patient Dosing and Sampling

The blood samples were taken during a study to investigate oral and intravenous pharmacokinetics of CyA in renal transplant patients; approximately 30 samples were taken from each of five patients over a 10-day period immediately posttransplant. The intravenous dose was given as a continuous infusion over 3 days at 7 mg/kg/day via a central venous line. This infusion was followed by an oral regimen of 8.5 mg, given as a freshly prepared emulsion with Caotina chocolate powder and cold milk, every 12 hr. A 12-hr oral profile was taken on day 8 for assessment of bioavailability.

Blood samples (10 ml) were taken by venipuncture into EDTA tubes and either processed immediately or stored at 4°C. Measurements of the blood-to-plasma concentration ratio (R), fraction unbound (fu), and hematocrit (H) were carried out within 24 hr of sampling.

Blood-to-Plasma Ratio (R) Measurements

The partitioning of CyA into erythrocytes was carried out at 37°C using a tritiated CyA tracer of specific activity 4000 dpm ng⁻¹ (supplied by Sandoz Ltd., Basel). The radiochemical purity of this material was >99%.

A 5-μl aliquot of radiolabeled CyA dissolved in methanol was freshly spiked into a clean tube and dried in a stream of oxygen-free nitrogen. The CyA was then dissolved and equilibrated with 4.0 ml of blood by incubation for at least 30 min in a 37°C water bath, with intermittent gentle mixing. The sample was then quickly centrifuged in buckets

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preheated to 37°C and the concentration of radioactivity in 180-μl aliquots of the plasma \((C_p^*)\) was determined by liquid scintillation counting in an LKB Rackbeta 1218 counter using a radioimmunoassay (RIA) Luma (Fisons, Loughborough) scintillator. The remaining radioactive plasma was used for determination of the fraction unbound (fu).

The total (whole-blood) concentration of radioactivity \((C_B^*)\) was obtained by counting 5-μl aliquots of the spiking solution, thus enabling the ratio \(C_B^*/C_p^*\) to be calculated. This ratio was taken to be the blood-to-plasma concentration ratio \((R)\) of unlabeled drug.

Fraction Unbound

The fraction of CyA unbound in plasma was determined by ultracentrifugation (3) using radiolabeled CyA in unadulterated plasma, spiked as described above. The binding measurements were all carried out at 37°C.

CyA Concentration in Plasma \((C_p)\)

This was measured by a high-performance liquid chromatographic (HPLC) method based on that of Carruthers et al. (6), using cyclosporin D as internal standard. Samples from each patient were analyzed simultaneously, and the intrabatch variability determined at a plasma concentration of 100 μg liter\(^{-1}\) was 4.1% (CV) and that at 1400 μg liter\(^{-1}\) was 5.0% (CV).

THEORY

Binding Isotherm

It is assumed that an equilibrium exists between unbound drug and drug bound to a single-site binding material associated with the erythrocyte, with the binding characterized simply by a dissociation constant \(K_d\). From mass-action considerations,

\[
K_d = \frac{C_u n P_t - C_{be}}{C_{be}} \quad (1)
\]

where \(C_u\) is the concentration of unbound drug, \(C_{be}\) is the concentration of drug bound to material in packed erythrocytes, \(P_t\) is the total concentration of binding material in packed erythrocytes, and \(n\) is the number of binding sites per unit of binding material. Rearrangement of Eq. (1) gives

\[
C_{be} = \frac{n P_t \cdot C_u}{K_d + C_u} \quad (2)
\]

Assuming that \(C_u\) is the same both inside and outside the erythrocyte, then the concentration of drug in packed erythrocytes, \(C_e\), is given by

\[
C_e = C_u + C_{be} \quad (3)
\]

Combining Eqs. (2) and (3) gives

\[
C_e = C_u + \frac{n P_t C_u}{K_d + C_u} \quad (4)
\]

If \(C_B\) is the total-blood concentration of drug, then by mass balance and considering a unit volume,

\[
C_B = C_p (1 - H) + C_e \cdot H \quad (5)
\]

where \(C_p\) is the concentration of drug in plasma and \(H\) is the hematocrit. Substitution of Eq. (4) into Eq. (5), replacing \(nP_t\) (total binding capacity in packed erythrocytes) by \(K_1\) and \(K_2\) by \(K_2\), gives

\[
C_B = C_p (1 - H) + H \left( C_u + \frac{K_1 C_u}{K_2 + C_u} \right) \quad (6)
\]

If the fraction unbound (fu) is independent of \(C_p\) (so that \(C_u = fu \cdot C_p\)), then the blood/plasma concentration ratio \((R)\) is derived from Eq. (6):

\[
R = \frac{C_B}{C_p} = (1 - H) + fu \cdot H \left( 1 + \frac{K_1}{K_2 + C_p} \right) \quad (7)
\]

The parameters \(K_1\) and \(K_2\) can thus be determined from the values of \(R, H, fu,\) and \(C_p\) associated with each sample. Once \(K_1\) and \(K_2\) have been determined, then \(R\) can be calculated from the three variables \(H, C_p\), and \(fu\) if they are either known or assumed to be constant.

The binding isotherm of Eq. (7) was fitted to the multivariate data \((R, H, fu,\) and \(C_p)\) for each patient data set using nonlinear least-squares regression, to yield estimates of \(K_1\) and \(K_2\).

At high CyA concentrations the whole-blood concentration becomes lower than the plasma concentration. The minimum value of \(R\) indicated by Eq. (7) is always \(<1\) and occurs as \(C_p \to \infty\):

\[
R_{\text{min}} = 1 - H (1 - fu) \quad (8)
\]

Estimation of Bioavailability

The following derivation for estimation of bioavailability for a drug with saturable erythrocyte binding is from a consideration of steady-state conditions.

Given a constant-rate infusion to steady state by both intravenous (iv) and oral (o) routes, then for measurements of drug in plasma,

\[
R_d(iv) = CL_d(iv) \cdot C_p(iv) \quad (9)
\]

\[
F_p R_d(o) = CL_d(o) \cdot C_p(o) \quad (10)
\]

and for measurements of drug in blood,

\[
R_d(iv) = CL_d(iv) \cdot C_d(iv) \quad (11)
\]

\[
F_b R_d(o) = CL_d(o) \cdot C_d(o) \quad (12)
\]

where \(R_d\) is the drug infusion rate; \(CL_d\) and \(CL_d\) are the clearances from plasma and blood, respectively; \(C_p\) and \(C_B\) are the steady-state plasma and blood concentrations, respectively; and \(F_p\) and \(F_b\) are the bioavailability values derived from plasma and blood data, respectively. Assuming that neither \(CL_d\) nor \(CL_d\) changes either with concentration or between treatments [i.e., \(CL_d(o) = CL_d(iv)\) and \(CL_d(o) = CL_d(iv)\)], then

\[
F_p = \frac{C_p(o)}{C_p(iv)} \cdot \frac{R_d(iv)}{R_d(o)} \quad (13)
\]

and

\[
F_b = \frac{C_B(o)}{C_B(iv)} \cdot \frac{R_d(iv)}{R_d(o)} \quad (14)
\]