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ORIGINAL ARTICLE

Clinical pharmacokinetics of Neoral in pediatric recipients of primary liver transplants

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Abstract Pediatric liver transplant recipients constitute a population characterized by a particularly unpredictable and poor bioavailability of cyclosporin (CyA). Even though several adult studies show that the new oral formulation of CyA, Neoral (NEO), produces better bioavailability and blood level predictability, few data describe its pharmacokinetics in children. We performed a complete analysis of the pharmacokinetics of NEO in ten small children after primary liver transplantation. Three pharmacokinetic profiles were set up with data obtained from tests taken during i. v. administration of CyA, after the first oral NEO dose, and after the last NEO dose before discharge from the hospital. The mean half-lives obtained were 8.1, 7.7, and 6.9 h, respectively, and the bioavailabilities were 22 % and 21 % for the first and last NEO doses. A large interpatient variability was observed. This was due, in part, to episodes of diarrhea that interfered with the pharmacokinetic evaluation and, in part, to the variability of post-transplant hepatic function. There was a good correlation between CyA trough levels and their related AUCs for both NEO profiles ($r = 0.93$ and $r = 0.74$, respectively). We conclude that, even though the pediatric OLT population remains more unpredictable than that of adults, NEO has a relatively rapid half-life and a remarkably improved bioavailability.

Key words Pediatric liver transplantation, Neoral, pharmacokinetics · Liver transplantation, pediatric, Neoral · Neoral, liver transplantation, pediatric · Pharmacokinetics, Neoral, pediatric liver transplantation

Introduction

The cyclosporin (CyA) present in the Sandimmun (SIM) formulation is erratically and incompletely absorbed after oral administration [8]. Inter- and intraindividual variabilities make oral dosing management very difficult. Pediatric patients who undergo orthotopic liver transplantation (OLT) constitute population characterized by a particularly unpredictable and poor bioavailability of CyA [2]. Some clinical studies on SIM have reported bioavailability values as low as 5 % in pediatric liver transplant recipients compared to 20 %–30 % in adult recipients [2, 7]. Furthermore, it is well known that pediatric patients metabolize and clear many drugs faster than adult patients and have higher volumes of distribution; therefore, higher doses may be required.

Neoral (NEO), the new oral formulation for CyA based on a microemulsion drug delivery system, has been shown to provide more efficient, stable, and predictable bioavailability than SIM in adult transplant recipients [4]. Our study was designed to analyze the pharmacokinetics (Pk) of NEO in small children after primary OLT and to investigate the dosing aspect further.

Data from this study should provide an indication of the dosage of NEO required for children.

Materials and methods

The present study was approved by the central Ethics Committee of the Saint Luc University Hospital in Brussels. A total of 15 pediatric patients were entered in an intention-to-treat (ITT) analysis after their parents had given informed consent. Five of the patients dropped out prematurely (one died and four were switched to other therapies) before all pharmacokinetic profiles had been completed. Because the results of the ITT analysis were similar to those of the per protocol analysis (n =10), any effect of the drop-outs on the results were minimal. The ten remaining children, who were similar in age (median 1.15 years, range 0.8–2.5 years), were selected for OLT for biliary atresia. Four of them received grafts from living related donors and the other six received reduced-size livers from cadaveric donors. Six hours after transplantation, all patients were given an i.v. dose of 2 mg/kg CyA per 24 h; this was subsequently adapted according to the desired trough level. The simultaneous administration of any drug that might interfere with CyA pharmacokinetics (e.g., erythromycin, ketoconazole, carbamazepine, etc.) was considered an exclusion criterion (with the exceptions of prednisolone and methylprednisolone). Once a stable level had been reached and maintained for at least 24 h and once the gastrointestinal tract activity had been restored, the i.v. CyA was discontinued in order to establish a wash-out period lasting 12 h. During this wash-out period, immunosuppression was maintained with corticosteroids and azathioprine. A first oral dose of NEO, of at least 5 mg/kg b.i.d., was administered after the 12-h wash-out. Both the i.v. and oral doses could be modified at any time if clinically indicated or according to specific whole blood trough levels (maintained within a target range of 100–400 µg/l) determined with a monoclonal antibody fluorescent polarization immunoassay (FPIA) on a TDx analyzer (Abbott Diagnostics, Chicago, Ill., USA). In parallel with this assay, all blood samples were further measured with a non-specific polyclonal FPIA assay in order to evaluate both the accumulation of CyA metabolites and the impact of the assay's specificity on pharmacokinetics. The laboratory participates in the U.K. Cyclosporin Quality Assessment Scheme (D. Holt, London).

Pharmacokinetic analysis was based on three profiles: Pk_1, Pk_2, and Pk_3. For the first profile (Pk_1), blood samples were taken at 0, 4, 8, 12, and 24 h after the onset of the i.v. CyA dose and every morning until the end of the infusion. In addition, blood samples were collected during the 12-h wash-out phase at 0, 4, 8, and 12 h. Pk_2 was determined following the first oral dose of NEO. Blood samples were taken at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 9, and 12 h after intake of this first oral dose. Finally, the last kinetic profile, Pk_3, was set up just before hospital discharge, when the patient was under steady-state conditions with improved hepatic function (e.g., increased clearance and increased hepatic first-pass effect). Blood was collected at the same times as for Pk_2.

The Pks were evaluated with standard methods for a noncompartmental analysis. C_{max}, the peak blood concentrations, and T_{max}, the time to attain C_{max}, were both determined by visual inspection of the data. The terminal elimination rate constant k_e was determined by linear regression of at least three data points from the terminal portion of the ln concentration-time plots, where $-k_e$ represents the slope. The area under the curve (AUC) after i.v. administration followed by the 12-h wash-out (AUC i.v.) was calculated with the linear trapezoidal rule up to the last measured blood concentration C_n and extrapolated to infinity by adding C_n/k_e (Eq. 1). Indeed, the wash-out phase was often not sufficient to assure a full return of the concentrations to the baseline.

$$AUC_{0\to\infty\ iv} = \sum_{i=1}^{n} \frac{(C_i+C_{i+1})}{2} \cdot (T_{i+1} - T_i) + C_n/k_{e\ iv} \quad (1)$$

C_i represents NEO blood concentrations and T_i, the time of sampling. The AUC obtained after the first oral administration, AUC_{po1}, was calculated similarly, but $C_n/k_{e\,iv}$, the extrapolated AUC resulting from the i.v. administration hidden under the blood concentration-time profile following the first oral dose, was substracted (Eq. 2).

$$AUC_{0\to\infty\ po1} = \sum_{i=1}^{n} \frac{(C_i+C_{i+1})}{2} \cdot (T_{i+1} - T_i) + C_n/k_{e\,po} - C_n/k_{e\ iv} \quad (2)$$

Finally, the AUC_{po2} obtained at steady state was calculated with the linear trapezoidal rule up to the last measured blood concentration drawn 12 h after the administration (Eq. 3).

$$AUC_{po2} = \sum_{i=1}^{n} \frac{(C_i+C_{i+1})}{2} \cdot (T_{i+1} - T_i). \quad (3)$$

Total body clearance (Cl) was calculated by dividing the dose by the corresponding AUC (Eq. 4). The elimination half-life, $t_{1/2}$, was estimated by dividing 0.693 by k_e (Eq. 5). The volume of distribution $V_{d\ area}$ was calculated by dividing Cl by k_e (Eq. 6), and the absolute bioavailability F was calculated with Eq. 7.

$$Cl = Dose/AUC \quad (4)$$

$$t_{1/2} = 0.693/k_e \quad (5)$$

$$V_{d\ area} = Cl/k_e \quad (6)$$

$$F = \frac{AUC_{po} \cdot Dose_{iv}}{AUC_{iv} \cdot Dose_{po}} \cdot 100. \quad (7)$$

The difference in the AUCs based on blood concentrations determined by the two assays (monoclonal and polyclonal antibodies) is assumed to be directly related to CyA metabolites in the blood and shows a certain crossreactivity with the antibody (Eq. 8).

$$AUC_{MET} = AUC_{polyclonal} - AUC_{monoclonal}. \quad (8)$$

Statistical differences were assumed to be significant when P was below 0.05 (unpaired t-test, assuming equal variances).

Results

The precision performances of the monoclonal FPIA method used routinely in the laboratory displayed values of 5.7 % and 4.2 % as interday and intraday coefficients of variations, respectively (n = 50). Clinical chemistry data corresponding to the Pk1, Pk2, and Pk3 periods are shown in Table 1 and illustrate the global improvement of hepatic function after transplantation. The mean i.v. period was 3.3 days and the mean i.v. dose during the last 24 h prior to discontinuation of the i.v. dose was low, 2.6 mg/kg. The mean NEO doses given

Table 1 Mean (range) clinical chemistry parameter values obtained at the time of the intravenous infusion (Pk_1), first oral administration (Pk_2), and steady state oral administration (Pk_3) of Neoral in ten pediatric primary liver transplant recipients (*AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* γ-glutamyl transferase)

Clinical chemistry parameters	Pk_1 Mean (Range)	Pk_2 Mean (Range)	Pk_3 Mean (Range)
Serum creatinine (μmol/l)	26 (9–44)	23 (9–35)	31 (26–40)
AST (IU/l)	1022 (146–5900)	69.4 (14–145)	27.8 (9–39)
ALT (IU/l)	928 (137–5720)	296 (28–1008)	35.5 (11–64)
GGT (IU/l)	74.8 (7–278)	141.6 (43–333)	83.1 (19–185)
Total bilirubin (μmol/l)	133 (44–318)	66 (20–214)	15 (7–37)

for the Pk_2 and Pk_3 were 5.3 and 10.9 mg/kg per 12 h, respectively. The children were discharged from the hospital after a mean stay of 23.3 days. At steady state, the mean calculated oral daily dose was about eight times the last i.v. daily dose. Table 2 shows Pk parameters obtained during the i.v. phase (Pk_1) and from both oral profiles (Pk_2, Pk_3). Mean i.v. clearance (Cl) and volume of distribution (Vd) were 6.2 ml/min per kg and 4.3 l/kg, respectively, with a resulting elimination half-life ($t_{1/2}$) of 8.1 h. Following the first oral dose, the mean Pk parameters observed were C_{max} 550 μg/l, T_{max} 2.5 h, trough level (C_{min}) 124 μg/l, Cl/F 48.7 ml/min per kg, Vd/F 28.9 l/kg, $t_{1/2}$ 7.7 h, and F 22 %. Even though large variations were reported during the last oral profile at steady state (Pk_3), no statistical differences were observed between Pk_2 and Pk_3, except for the dose and its related parameters (AUC and C_{max}). The mean value of the peak concentration was 1002 μg/l at steady state. The concentration versus time profiles are illustrated in Fig. 1 at steady state (Pk3). No positive correlation was observed between the doses and the trough levels, or between the age and Pk parameters. The origin of the organ transplanted (living related vs cadaveric donor) apparently did not affect the Pk parameters. However, a good correlation was found between the AUCs and their related trough levels for both Pk_2 and Pk_3, with correlation coefficients of 0.93 (Fig. 2) and 0.74, respectively.

Two children experienced severe episodes of diarrhea during the last Pk phase (Pk_3), most likely affecting the pharmacokinetic parameters and therefore, contributing to the high variability of the data. For this reason, the data were evaluated again after exclusion of the data in the last phase for these two children as shown in Table 2, resulting in a lower standard deviation, but without markedly affecting the data.

The accumulation of CyA metabolites as a factor of hepatic cholestasis is illustrated by the mean AUC_{MET} values of 9750 and 4276 μg/l obtained during the immediate post-transplant period (Pk1) and at the steady state before discharge from the hospital (Pk3), respectively. Pharmacokinetic parameters obtained with the nonspecific polyclonal immunoassay were significantly different from the parameters of monoclonal immunoassay for the C_{max} and AUC values ($P < 0.01$), as expected. Cl and Vd displayed lower values (Cl 4.3 ml/min per kg for Pk1, $P < 0.01$; Vd 3.3 l/kg for Pk1, $P < 0.05$). The F values were slightly higher (28 % and 25 % for Pk2 and Pk3, respectively, $P = 0.05$; but no statistical differences were observed for $t_{1/2}$ values. Furthermore, the correlation observed between the AUC and the trough levels remained high, with r values of 0.959 and 0.825 for Pk2 and Pk3, respectively.

Discussion

Most of the Pk parameters reported in the literature were estimated for adults. Cyclosporin (SIM) bioavailability after OLT in pediatric patients has been reported to be very low by some authors: less than 5 % in the im-

Table 2 Mean (SD) Pk parameters after intravenous infusion (Pk_1), first oral administration (Pk_2), and steady state oral administration (Pk_3) of Neoral in ten pediatric primary liver transplant recipients

Pk parameters	Pk_1 Mean (SD)	Pk_2 Mean (SD)	Pk_3 Mean (SD)	
Dose (mg/kg per day)	2.6 (0.6)	2 × 5.3 (1.7)	2 × 10.9 (3.3)	
C_{max} (μg/l)	468 (333)	550 (442)	1002 (422)	1131 (327)[a]
C_{min} (μg/l)		124 (96)	243 (156)	
T_{max} (h)	59 (35)	2.5 (2.5)	2.7 (2)	
$t_{1/2}$ (h)	8.1 (2.5)	7.7 (2.8)	6.9 (2.9)	
AUC (μg · h/l)	21986 (5735)	3372 (2138)	5827 (2493)	6702 (1835)[a]
r (AUC vs C_{min})		0.93	0.74	
Cl (ml/min per kg)	6.2 (2.2)	48.7 (65.8)	42.3 (34.2)	32.9 (21.1)[a]
Vd (l/kg)	4.3 (1.3)	28.9 (32.2)	24.3 (24.3)	16.8 (6.9)[a]
F (%)		22 (12)	21 (12)	24 (11)[a]
AUC_{MET} (μg · h/l)	9750 (5087)		4276 (2503)	

[a] Corrected Pk3 data after removal of two patients for acute diarrhea episodes

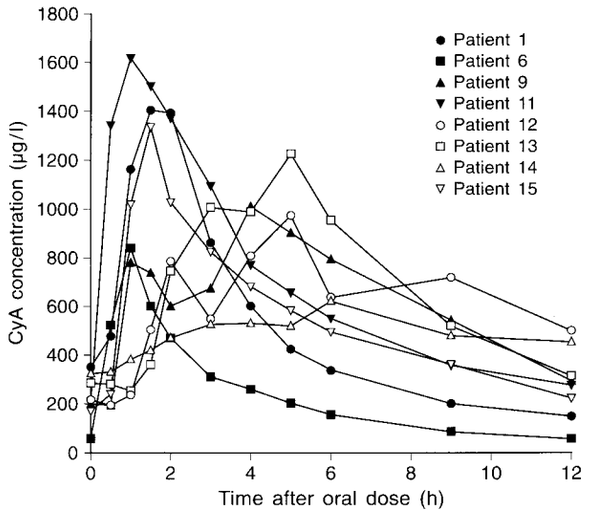

Fig. 1 Cyclosporin concentrations versus time profiles ($n = 8$) obtained after Neoral administration at steady state Pk_3 with the monoclonal whole blood FPIA method

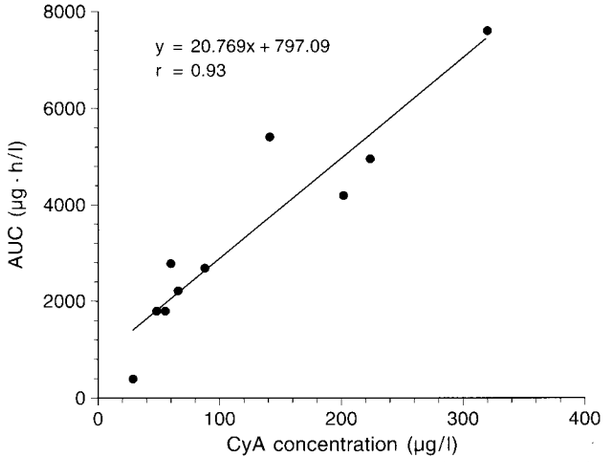

Fig. 2 Correlation between the area under the time-concentration curves (AUC) and their corresponding whole blood cyclosporin trough levels ($n = 10$) determined at the first oral dose of the Neoral formulation

mediate postoperative period [2] or 7.8 % [7], compared to a mean of 27 % in adults [2, 8]. Dependence upon bile salts made the bioavailability of SIM after OLT even more incomplete and variable. The correlation between trough levels and the AUC was poor with SIM ($r = 0.6$). This is considered handicap since AUC or F appears to correlate more strongly with organ rejection than with trough levels [5, 6], but the parameters are much more difficult to obtain than the trough level.

The development of NEO has provided improved and more consistent absorption characteristics, resulting in both a more predictable pharmacokinetic profile and a lower food and bile dependence relative to SIM. In the pediatric OLT population, which is considered a high-risk group for malabsorption, we have observed a significant improvement in the bioavailability, reaching values greater than 20 %, as well as an improvement in the predictability of the AUC from the trough levels. Whereas a lower F value was expected at the steady state due to the improvement of the first-pass effect, no significant difference was observed between the two bioavailabilities (at day 3 and day 23).

In a previous study comparing SIM and NEO Pk in two OLT children with compromised CyA absorption (for chronic rejection and diarrhea secondary to proximal bowel resection), we demonstrated a dramatic improvement in the bioavailability with NEO, increased by factors greater than 5 after correcting for the dose [1]. In a recent Pk analysis of NEO in de novo pediatric liver transplant recipients, Dunn et al. [3] also report an improvement in bioavailability with NEO (37 %) compared with SIM (24.7 %). However, the pediatric population they analyzed differed more in terms of age (range 0.5–11 years, with a mean age more than twice as high as in our population). Our data display considerable interindividual differences, partly due to diarrhea episodes and partly due to the variability in hepatic function (Table 1). It is not surprising to observe a trend toward a correlation between total bilirubin levels and elimination half-life ($r = 0.64$). Furthermore, the lowest serum AST values correspond to the patients with the highest CyA clearance and conversely ($r = 0.73$).

Different specific analytical methods, FPIA [1], high-performance liquid chromatography [2, 7], and radioimmunoassay [3] have been used in previous pharmacokinetic reports. In the case of immunoassays, one should keep in mind that a few CyA metabolites may still present a cross-reactivity with the monoclonal antibody (particularly the FPIA, resulting in blood concentrations 5 %–25 % higher than with HPLC) and may, therefore, slightly interfere with the results. However, in addition to the specificity, another important analytical quality needed for such a study is reproducibility. We have seen that the FPIA method is currently one of the most reproducible assays, displaying coefficient of variation values much lower than those with HPLC. Even though important differences were noted in the comparative pharmacokinetic data obtained between polyclonal and monoclonal immunoassays, clearly justifiying the use of specific measurements for pharmacokinetic evaluations, no significant differences were observed for the $t_{1/2}$ values or for the AUC/trough level correlation.

We conclude that the new microemulsion formulation of CyA (NEO) is able to enhance the absorption of CyA after pediatric OLT, despite the young age and the impaired liver function of the patients. Even though

there is an improvement in the bioavailability of CyA after the low i.v. daily dose used in our study (2.6 mg/kg). at the steady state before hospital discharge, the oral daily dose was about eight times the daily i.v. dose. This oral daily dose could be divided into two equal doses of about 10 mg/kg to be administered every 12 h or, if desired, into three equal doses of about 7 mg/kg to be administered every 8 h.

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