Background and Objectives. A major problem encountered during oral cyclosporin-A (CsA) administration to prevent acute graft-versus-host-disease (GVHD) after allogeneic bone marrow transplantation (allo-BMT) is its irregular pharmacokinetics. The aim of this study was to evaluate the pharmacokinetics of Neoral™, a new water-free microemulsion formulation of CsA.

Design and Methods. Eighteen patients aged over 18 were enrolled into the study. When able to eat normally after allo-BMT, patients received CsA orally and after 4 days a 12-hour CsA pharmacokinetic profile was constructed. Three patients received Sandimmune™ 10 mg/kg/day, 5 patients received Neoral™ 7.5 mg/kg/day and 10 patients Neoral™ 5 mg/kg/day. CsA concentration was analyzed on whole blood by high-performance liquid chromatography (HPLC).

Results. Neoral™ showed concentration-time profiles characterized by a smooth and faster rise to the C max value compared to that produced by Sandimmune™. The comparison between pharmacokinetic parameters obtained in patients receiving Neoral™ 5 mg/kg/day or 7.5 mg/kg/day showed a proportional increase of the AUC (4776±1084 vs. 7746±2006 ng/mL h) and C max (1027±203 vs. 1514±231 ng/mL). In all patients to whom 7.5 mg/kg/day of Neoral™ were given, C trough levels were always above the threshold of 200 ng/mL.

Interpretation and Conclusions. Our data suggest that oral administration of Neoral™ 7.5 mg/kg/day early after allo-BMT may represent an appropriate dose resulting in adequate CsA C trough levels without significant renal toxicity.

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Key words: allogeneic bone marrow transplantation, cyclosporin-A, immunosuppression, graft-versus-host disease
in daily clinical practice, we also evaluated the AUC. This latter is, in fact, more informative about the real exposure to CsA and represents the most appropriate parameter to predict graft rejection and CsA-related toxicity after solid organ transplantation.14, 15 Unfortunately, the high number of samples usually required to perform a conventional determination of AUC limits the clinical applicability of this parameter. Previous experience in kidney transplant recipients showed that the reproducible pharmacokinetics observed after Neoral™ allowed an innovative three point sampling strategy to be used to predict the CsA AUC.16 We, therefore, decided to apply the same approach to allo-BMT patients.

Design and Methods

Patients

Eighteen consecutive non-randomized patients (7 females and 11 males) aged over 18 were enrolled into the study. Seven patients had chronic myelogenous leukemia, 5 patients acute myelogenous leukemia, 4 patients acute lymphoblastic leukemia, 1 patient multiple myeloma and 1 patient myelodysplastic syndrome. The conditioning regimens were as follows: busulfan/cyclophosphamide (10 patients), total body irradiation/cyclophosphamide (3 patients), total body irradiation/melphalan (3 patients), thiopeta/cyclophosphamide (1 patient), and busulfan/melphalan (1 patient). Thirteen patients received peripheral blood progenitor cells (PBPC) from an HLA-identical sibling, 1 patient received bone marrow (BM) from an HLA-identical sibling and 4 patients received BM from an HLA-identical unrelated donor. All patients were regularly followed at the Bone Marrow Transplant Unit at Divisione di Ematologia, Ospedali Riuniti di Bergamo. The study protocol was described in detail to all patients before admission and informed consent to the study was obtained in each instance.

Study schedule

For all patients the prophylaxis for acute GVHD consisted of intravenous CsA (1 mg/kg/day over 24 hours as a continuous infusion) starting the day before the infusion of BM or PBPC.17 In addition, all patients received intravenous infusion of MTX (15 mg/m² on day +1 and 10 mg/m² on day +3, +6 and +11). When patients were able to eat normally, usually between day 20 and 30, they were changed to the oral CsA administration and after four days, a 12-hour CsA pharmacokinetic profile was measured after the morning dose of CsA. The pharmacokinetics was based on analysis of blood samples collected from the antecubital vein just before the dose (C₀ or Ctrough), and 30 minutes as well as 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours after drug administration. During the same period 3 patients received Sandimmune™ 10 mg/kg/day, 5 patients received Neoral™ 7.5 mg/kg/day and 10 patients received Neoral™ 5 mg/kg/day. The planned dose of CsA was administered in two divided doses every 12 hours. During the pharmacokinetic study, all patients were monitored daily for vital signs and twice weekly for laboratory variables (renal and liver function tests). After discharge patients were followed twice weekly for one month and the CsA dose was modified to maintain the whole blood CsA Ctrough within 200 and 400 ng/mL. For all patients, in case of acute GVHD occurrence, intravenous CsA was re-established at the dose of 3 mg/kg/day in association with methylprednisolone (2 mg/kg/day).

CsA pharmacokinetic evaluation

Blood samples were analyzed by high-performance liquid chromatography (HPLC) as previously described.16 The blood concentration-time profile of CsA was recorded for all patients together with Ctrough, C₁₂, Cmax, and the time of maximum observed concentration (Tmax). The blood concentration-time profile of CsA was recorded after the morning dose of CsA. The AUC from C₀ to the last sampling point (12 hr) (AUCₐ →ₜ₁₂) was calculated by the trapezoidal rule. Predicted AUC after Neoral™ administration was estimated using a three-point sampling strategy (sampling points 0, 1, and 3 h), as previously described.16

Results and Discussion

When the Neoral™ formulation became available, we treated 3 initial patients with 10 mg/kg/day in two divided doses maintaining the 1 to 1 conversion with the conventional Sandimmune™ formulation according to the manufacturer’s instruction. However, in these patients we noticed that CsA Ctrough levels measured during the early days after the beginning of Neoral™ administration were usually very high, consistently above the value of 500 ng/mL. The last of these patients developed clinical and laboratory evidence of thrombotic thrombocytopenic purpura (TTP) concomitant with a CsA Ctrough of 1,119 ng/mL and this is in line with reports on a possible role of CsA overexposure.18, 19 From this experience we decided to perform a pharmacokinetic study in order to optimize the dose of Neoral™.

The whole blood 12-h concentration profiles recorded for patients given either Sandimmune™ or Neoral™ are shown in Figure 1. The profiles obtained for patients given Neoral™, regardless of the dose employed (5 mg/kg/day, Panel A or 7.5 mg/kg/day, Panel B) showed a smooth and faster rise to the Cmax value compared to that given by Sandimmune™ (10 mg/kg/day, Panel C). Although, we studied few cases with Sandimmune™, our results concerning the poor pharmacokinetic profiles of this CsA formulation are in keeping with those reported in the literature.2, 5, 7 We, therefore, suggest that a more consistent CsA concentration time profile is obtained after Neoral™ administration in allo-BMT patients as previously reported for solid organ transplant recipients.10, 11, 20 The comparison between pharmacokinetic parameters obtained in patients receiving Neoral™ 5 or 7.5 mg/kg/day, showed a 50% increase of AUC (4776±1084 vs. 7746±2006 ng/mL h) and Cmax (1027±203 vs. 1514±231 ng/mL), and a 80-90% increase of both Ctrough (186±80 vs. 348±90 ng/mL) and C₁₂ (184±103 vs. 325±109 ng/mL) (Table 1). In all patients to whom 7.5 mg/kg/day of Neoral™ were giv-
Neoral™ in allogeneic bone marrow transplantation

en, C_{trough} as well as C_{12} levels were always above the threshold of 200 ng/mL.

The existence of a tight relationship between CsA concentration and appropriate GVHD prophylaxis has long been recognized.2-7 Most data reported in the literature have been obtained by the evaluation of serum or plasma CsA C_{trough} using HPLC or radioimmunoassay (RIA) either with polyclonal or monoclonal antibodies.2-7. In particular, Yee and co-workers suggested that a serum CsA C_{trough} between 200 and 400 ng/mL measured with a polyclonal-RIA was associated with a lower risk of acute GVHD.3 Nowadays, however whole blood CsA concentrations evaluated by a specific monoclonal antibody are usually recommended in routine clinical activity21 and it is difficult to compare CsA concentrations evaluated by different methods and in different matrices. In general, CsA concentration in whole blood is almost double that in serum,21,22 and C_{trough} levels measured with RIA-methods tend to be higher (ranging from 1.37 to 1.5 times) than those measured by HPLC.23,24 Putting these data, we arbitrarily suggest that C_{trough} levels of 348±90 ng/mL obtained after Neoral™ 7.5 mg/kg/day in this study can be compared to the therapeutic range of 200-400 ng/mL (measured on serum with a polyclonal-RIA) reported by Yee et al.3 CsA C_{trough} was also evaluated in ten additional patients, followed outside the pharmacokinetic study, to whom an oral dose of Neoral™ 7.4±0.5 mg/kg/day was administered in the early period after transplantation. In these patients we obtained mean C_{trough} levels of 513±244 ng/mL (measured on whole blood with a monoclonal-RIA) 4-6 days after beginning Neoral™. In four of these patients CsA C_{trough} was higher than 500 ng/mL and consequently the dose of CsA was reduced to 5 mg/kg/day and after that the C_{trough} was 338±71 ng/mL. The suggested dose of 7.5 mg/kg/day of Neoral™ as a starting oral dose of CsA in the setting of allo-BMT is in keeping with data recently reported by Parquet et al.25 who suggest that for patients receiving CsA 3 mg/kg by continuous i.v. infusion, the appropriate starting oral dose of Neoral™ is twice the last i.v. dose.

Acute and chronic renal dysfunction are frequently encountered during CsA administration and these are usually dose-dependent.7 In Table 2, we summarize the serum creatinine levels measured at different time

Table 1. CsA pharmacokinetic parameters in allogeneic bone marrow transplant recipients after Sandimmune™ or Neoral™ administration.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>CsA formulation</th>
<th>Dose (mg/kg/day)</th>
<th>AUC (ng.h/mL)</th>
<th>C_{0} (ng/mL)</th>
<th>C_{trough} (ng/mL)</th>
<th>C_{12} (ng/mL)</th>
<th>C_{max} (ng/mL)</th>
<th>T_{max} (h)</th>
</tr>
</thead>
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<tr>
<td>3</td>
<td>Sandimmune™</td>
<td>10</td>
<td>11227 ± 507</td>
<td>628 ± 118</td>
<td>412 ± 198</td>
<td>1735 ± 176</td>
<td>4.7 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Neoral™</td>
<td>5</td>
<td>4776 ± 1084</td>
<td>186 ± 80</td>
<td>184 ± 103</td>
<td>1027 ± 203</td>
<td>2.1 ± 1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Neoral™</td>
<td>7.5</td>
<td>7746 ± 2006</td>
<td>348 ± 90</td>
<td>325 ± 109</td>
<td>1514 ± 231</td>
<td>1.3 ± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Pharmacokinetic profiles after administration of CsA Neoral™ or Sandimmune™ in allo-BMT recipients. When able to eat normally, the patients were given the CsA orally. Four days later, CsA pharmacokinetic profile was measured after the morning dose of drug. Ten patients received Neoral™ 5 mg/kg/day (Panel A), 5 patients received Neoral™ 7.5 mg/kg/day (Panel B) and 3 patients received Sandimmune™ 10 mg/kg/day (Panel C). The shaded area represents ± 1 SD.
points in patients receiving either Sandimmune™ or Neoral™. In no patients to whom 5 or 7.5 mg/kg/day of Neoral™ were given, did we observe an increase of the serum creatinine level above the threshold of 2 mg/dL within the period of observation.

The evidence that the AUC is a more accurate indicator of total CsA drug exposure in solid organ transplant recipients14,15 and the consistency of Neoral™ CsA profiles prompted us to investigate whether an innovative three-point sampling strategy early after CsA dosing (at 0, 1, and 3 h),16 could be used to predict AUC in allo-BMT patients. As shown in Figure 2, the predicted AUC calculated by this strategy was accurate (4879±1242 ng/mL h after 5 mg/kg/day and 7445±1364 ng/mL h after 7.5 mg/kg/day) and not statistically different from the conventionally evaluated areas (4776±1084 ng/mL h after 5 mg/kg/day and 7746±2006 ng/mL h after 7.5 mg/kg/day). Moreover, the calculated error (measured as: AUC measured/AUC measured x100) was very modest with a mean value of 0.81±7.55% (range 14.4±14.3%).

Taken together these data suggest that the use of 7.5 mg/kg/day of Neoral™ may represent an appropriate dose during the early oral CsA administration in patients undergoing allo-BMT, resulting in adequate CsA trough levels without significant acute renal toxicity. Whether this significantly prevents the chronic renal toxicity induced by CsA still remains to be demonstrated. Finally, the easy determination we proposed for CsA AUC could be validated within prospective clinical studies designed to evaluate the ability of this pharmacokinetic parameter to predict the risk of acute GVHD and CsA-related toxicity.

Contributions and Acknowledgments
GD and FG contributed equally to this work. GD, FG and AR designed the study and prepared the manuscript. FG and RC carried out HPLC measurements. NP, GR and TB contributed to the discussion and approved the final version to be submitted. We wish to thank all the nursing staff for their excellent support in the clinical management of the Bone Marrow Transplant Unit.

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Disclosures
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Redundant publications: no substantial overlapping with previous papers.

Manuscript processing
This manuscript was peer-reviewed by two external referees and by Prof. Jorge Sierra, who acted as an Associate Editor. The final decision to accept this paper was taken jointly by Prof. Sierra and the Editors. Manuscript received November 17, 2000; accepted February 14, 2001.

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### Table 2. Serum creatinine levels in allogeneic bone marrow transplant recipients receiving CsA.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>CsA formulation</th>
<th>Dose (mg/kg/day)</th>
<th>S-creatinine (mg/dL) day 0</th>
<th>S-creatinine (mg/dL) day +4</th>
<th>S-creatinine (mg/dL) day +14</th>
<th>S-creatinine (mg/dL) day +30</th>
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</thead>
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<tr>
<td>3</td>
<td>Sandimmune™</td>
<td>10</td>
<td>1.1±0.18</td>
<td>0.8±0.14</td>
<td>1.17±0.21</td>
<td>1.4±0.53</td>
</tr>
<tr>
<td>10</td>
<td>Neoral™</td>
<td>5</td>
<td>0.83±0.18</td>
<td>0.92±0.16</td>
<td>0.98±0.17</td>
<td>0.96±0.18</td>
</tr>
<tr>
<td>5</td>
<td>Neoral™</td>
<td>7.5</td>
<td>0.82±0.18</td>
<td>0.95±0.06</td>
<td>1±0.16</td>
<td>1.12±0.33</td>
</tr>
<tr>
<td>10*</td>
<td>Neoral™</td>
<td>7.4±0.5</td>
<td>1.06±0.23</td>
<td>1.11±0.18</td>
<td>1.11±0.42</td>
<td>1.04±0.38</td>
</tr>
</tbody>
</table>

* Patients evaluated outside the pharmacokinetic study.
Potential implications for clinical practice

The reproducibility of Neoral™ pharmacokinetics and the predictable AUC calculated by the simple three-point method may offer the opportunity to evaluate the significance of the AUC parameter in GVHD prevention in prospective studies.

References


