COMPARISON OF BIOAVAILABILITY AND METABOLISM WITH TWO COMMERCIAL FORMULATIONS OF CYCLOSPORINE A IN RATS

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(Received November 7, 2001; accepted February 26, 2002)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

The bioavailability and metabolism of cyclosporine A (CsA) capsules were compared with two bioequivalent (Food and Drug Administration approved) preparations in rats. Two groups of Wistar-Kyoto rats were given 10 mg/kg q.d. of Sandimmun Neoral (NEO), Novartis Pharma, and CsA (United States Pharmacopeia modified), Eon Labs (EON), as capsules dissolved in water by oral gavage. After reaching steady-state (SS), rats were euthanized 2, 4, 8, 12, and 24 h after dosing. Parallel to this investigation, a single dose (SD) study was also performed. CsA and CsA metabolite concentrations of AM1, AM4N, and AM9 were determined by high-performance liquid chromatography in kidney, whole blood, and urine. The bioavailability of EON was 15% lower (area under the curve (AUC)SS blood CsA, 27.9 ± 3.69 mg · h/l) in the blood and 40% lower (AUCSS kidney CsA, 136.2 ± 21.2 mg · h/l) in the kidney in contrast to NEO (AUCSS blood CsA, 32.1 ± 4.32 mg · h/l and AUCSS kidney CsA, 220.8 ± 29.5 mg · h/l). In contrast, the plasma AM4N level was significantly elevated in group receiving EON (AUCSS blood AM4N, 4.1 ± 0.42 mg · h/l) compared with the other group treated with NEO (AUCSS blood AM4N, 2.9 ± 0.39 mg · h/l). In the kidneys, no significant differences were observed concerning the AM4N concentrations of NEO (AUCSS kidney AM4N, 11.8 ± 1.87 mg · h/l) versus EON (AUCSS kidney AM4N, 12.1 ± 2.14 mg · h/l), but AM1 was increased (AUCSS kidney AM1, 54.3 ± 11.2 mg · h/l) in comparison to NEO (AUCSS kidney AM1, 20.5 ±/− 3.56 mg · h/l). Furthermore, EON produced a larger amount of AM4N in the urine (5.8 ± 0.85 μg/24h versus 2.2 ± 0.95 μg/24h). Similar results were obtained with the SD study. Although the clinical consequences of our results remain at present unknown, the data suggest differences in CsA disposition that may affect drug efficacy and safety and merit further investigation in humans.

CsA is still the center of immunosuppressive regimen after solid organ transplantation and is further employed in the treatment of autoimmune disorders. It is known that CsA possesses very high inter- and intraindividual pharmacokinetic variability (Kahan et al., 1996) if orally applied in a lipophilic solvent (e.g., olive oil). Absorption in the small intestine, mainly by passive diffusion (LeGrue et al., 1983), is highly bile-dependent when using such an oil-based formulation. Necessary emulsification of the crude oil-in-water droplet mixture formed by intestinal digestion (Drewe et al., 1992) varies form the presence of food, composition of the gastric fluids and thus enhances oral bioavailability. The first microemulsion pharmaceutical research was focused on a microemulsion preconcentrate formulation with self-emulsifying properties, which immediately forms a microemulsion upon contact with the aqueous gastrointestinal fluids and thus enhances oral bioavailability. The first microemulsion product, Sandimmun Neoral, has improved immunosuppressive efficacy due to a better oral bioavailability, lower pharmacokinetic variability, and better dose-linearity compared with the former Sandimmun Neoral (Dunn et al., 2001). But the need to reduce costs in the health system in nearly every country leads to generic substitutions of the original products. Different generic manufacturers now produce CsA formulations with modified bioavailability (CsA United States Pharmacopeia modified; approved as AB-rated by the Food and Drug Administration) for generic substitution of the original product Sandimmun Neoral. In this study, we concentrated on evaluating the bioequivalence of the generic EON compared with NEO in rats primarily by comparing the occurrence of CsA in whole blood, kidneys, and urine and the bioavailability of CsA in the kidneys. It is known that small changes in the pharmacological inactive excipients of a galenic formulation can profoundly alter drug absorption
Since their appearance is time and tissue dependent. Furthermore, findings to those transplanted patients showing elevated CM levels, et al., 1990). However, it is nearly impossible to extrapolate these observations to the benefits of these CM. The activity of CM has been restricted to in vitro and animal tests (Fahr et al., 1990). The possible activity or renal side effects of these CM are still controversial. Experiments to determine immunosuppressive or toxic activity of CM have been restricted to in vitro and animal tests (Fahr et al., 1990). However, it is nearly impossible to extrapolate these findings to those transplanted patients showing elevated CM levels, since their appearance is time and tissue dependent. Furthermore, possible additional or synergistic effects have to be considered as well. AM4N is mainly generated by the gastrointestinal CYP3A in humans and rats (Schwinghammer et al., 1991), therefore we focused on the detection of this CM for the comparison of the two CM preparations and discussed its possible role as a marker for the extent of CsA absorption. We compared CsA and CM (AM1, AM4N, and AM9) pharmacokinetics in whole blood and in the kidneys after single dose and under steady-state conditions between NEO and EON. Additionally, CsA and CM recovery in the urine were obtained from a 24-h postdose collection. For the detection of possible renal damage, the serum creatinine levels were determined as well.

Materials and Methods

Experimental Design. Seventy adult normotensive Wistar-Kyoto rats (Charles River, Sulzfeld Germany) weighing 250 to 280 g were randomized into two groups and each of these groups subdivided into five groups containing seven animals each. The capsules of NEO (Novartis Pharma Ltd., Basel, Switzerland) and EON (Eon Labs Manufacturing, Inc., Laurelton, NY) were dissolved for peroral application by a gavage. The emulsions (containing 2 mg/ml) were freshly prepared each day; one capsule of the investigated products (containing 100 mg of CsA each) was put into a graduated glass flask, dissolved by adding sterile isotonic sodium chloride solution up to 50 ml, and mixed on a magnetic stirrer for 30 min at room temperature. Group 1 was given NEO and group 2 received EON; a rat weighing 250 g received 1.25 ml of the prepared emulsion, corresponding to 10 mg/kg·day. On day seven, six rats of each main group were held in metabolic cages to obtain urine over 24 h. On day eight, one rat of each subgroup was killed before the final dosing (t = 0) to obtain the trough levels (C₀). All remaining rats in the five subgroups were euthanized after inhalation of sevoflurane 2, 4, 8, 12, and 24 h after the final dosing for tissue collection.

The SD experiment was performed in the same manner but without predose determination of trough levels. Wistar-Kyoto rats (n = 60) were randomly divided into five groups containing six animals each. After single dosing of 10 mg/kg, the animals were killed at the same timepoints as previously described. One group was held in metabolic cages for 24 h to obtain the urine. CsA concentration and the concentration of the CM were determined in whole blood, kidney, and also in the urine by high-performance liquid chromatography (HPLC) in both study designs.

Sample Preparation. Tissue samples were finely cut with a razor blade on ice, weighed to obtain 100-mg samples, and homogenized with an ultra-turrax phy (HPLC) in both study designs.

HPLC Conditions. The method was adapted from Brozmanova et al. (2000). The chromatographic equipment consisted of pump LC 10AT, autotampler SIL 10A, UV detector SPD 10AV set at 205 nm, and Class 10 integration software (Shimadzu Europe, Duisburg, Germany). Separation was performed using a Luna phenylhexyl column (i.d. 150 × 6.4 mm; Phenomenex, Aschaffenburg, Germany) with acetonitrile/methanol/water (200:80:140, v/v/v) as eluent. At a flow rate of 1 ml/min (column temperature 75°C), AM9

**Fig. 1.** Concentration time curves of CsA and CM (NEO and EON) in whole blood are shown under SS and SD conditions after peroral administration of 10 mg/kg·day.

Panel A, CsA: NEO SS; EON SS; NEO SD; EON SD. Panels B and C, CM: AM1 NEO; AM1 EON; AM4N NEO; AM4N EON; AM9 NEO; AM9 EON. Results are expressed as means ± S.D. *, P < 0.05; ***, P < 0.001.
eluted at 6.1 min, AM1 at 6.8 min, AM4N at 8.4 min, CsA at 13.3 min, and finally cyclosporine D (internal standard) at 16.7 min.

Statistical Evaluation and Pharmacokinetic Analysis. Pharmacokinetic parameters were determined by standard noncompartmental analysis. Maximum plasma concentration (C\text{max}) and time of C\text{max} (t\text{max}) were noted directly. The area under the plasma concentration-time curves (AUC \text{0-T last}) from 0 to 24 h of CsA and AM1, AM4N, and AM9 were calculated by the linear trapezoidal rule.

C\text{max} and AUC obtained from EON were compared with NEO by analysis of variance with multiple comparison followed by the Student’s t test. Blood SS (a), blood SD (b), kidney SS (c) and kidney SD (d) were compared separately. P \text{values} < 0.05 were considered significant and indicated in the tables as superscript letters (a, b, c, d, respectively).

Results

Rats treated with EON showed a nonsignificant lower AUC (about 15%) and a different absorption profile of CsA in whole blood compared with NEO in the rat, both under SS and SD conditions (Fig. 1A; Table 1). In contrast to the CsA levels, the AM4N concentration was significantly elevated in the blood (Fig. 1B) in the EON group compared with NEO, again under both conditions. The AM4N whole blood levels following NEO administration were in the range described previously by Kovarik et al. (1994). Furthermore, under SS conditions, the profile of AM1 was altered but not significantly increased (Fig. 1B; Table 1), whereas the AM9 levels did not differ at all between both preparations. There were no observable changes in the CM values under SD conditions, except AM4N (Fig. 1C; Table 1).

The described differences in whole blood were even more pronounced in the kidneys. The AUC (CsA) of EON was about 40% lower (Fig. 2A; Table 2) than the AUC of NEO, whereas the AM4N levels in the kidneys interestingly were not affected by this circumstance (Fig. 2B; Table 2). In addition to our findings in the blood, the AM1 concentration was significantly higher in the EON SS group, whereas the other metabolites did not differ between NEO and EON under SS conditions. There were no differences observed concerning the metabolites under SD conditions (Fig. 2C; Table 2) between NEO and EON. The only metabolite recovery that was increased in the urine was that of AM4N in the SS as well as in the SD experiment (Fig. 3, A and B), whereas the CsA excretion over 24 h did not show any observable alteration. No significant changes were observed in the serum creatinine levels of all groups (NEO SD, 0.41 ± 0.08 mg/dl; EON SD, 0.45 ± 0.07 mg/dl; NEO SS, 0.50 ± 0.13 mg/dl; EON SS, 0.51 ± 0.11 mg/dl).

Discussion

Our experimental findings in rats show significant differences in the CsA absorption rate, bioavailability, and CM profiles between two approved bioequivalent preparations, NEO and EON, both under SD
human transplant patients. Nevertheless, both preparations were dissolving them before oral gavage, and studied only a short period; this could also be due to accumulation of CM. SS compared with SD indicate an increased first-pass metabolism, but these mechanisms. However, it appears that higher CM levels under expression (CYP3A family) and -activity] are necessary to elucidate metabolism rate of CsA in vivo, but further studies [e.g., on enzyme-Freijs and Karlsson (1994). They reported a dose dependence on the tem in the intestinal mucosa or in the liver as proposed by Lindberg-AM4N and possibly indicates a saturable first-pass metabolism sys-absorption process for NEO probably produced smaller amounts of tissues were more pronounced and potentially serious. The faster AUC of CsA was not significant, the results obtained in the kidney and SS conditions. Although the observed difference in the blood and SS conditions. Although the observed difference in the blood AUC of CsA was not significant, the results obtained in the kidney tissues were more pronounced and potentially serious. The faster absorption process for NEO probably produced smaller amounts of AM4N and possibly indicates a saturable first-pass metabolism system in the intestinal mucosa or in the liver as proposed by Lindberg-Freijs and Karlsson (1994). They reported a dose dependence on the metabolism rate of CsA in vivo, but further studies [e.g., on enzyme-expression (CYP3A family) and -activity] are necessary to elucidate these mechanisms. However, it appears that higher CM levels under SS compared with SD indicate an increased first-pass metabolism, but this could also be due to accumulation of CM. Since we investigated rats, altered the original products by dissolving them before oral gavage, and studied only a short period of time, it is not possible to draw conclusions concerning either nephrotoxic effects of CM or their impact on graft survival in human transplant patients. Nevertheless, both preparations were tested under identical experimental conditions. Furthermore, our results could be masked by the tremendous amount of variability in CsA bioavailability in transplanted patients in the clinical setting. The possible clinical consequences of the use of generic CsA will become evident after several years and after multiple switching between different generic formulations. Therapeutic drug monitoring (TDM) for CsA after solid organ transplant mostly uses the CsA C₀ levels as an input for further dose adjustment. Our data show, that in spite of the inequivalence of metabolism, absorption rate, and differences in CsA kidney levels, the C₀ levels in the blood were not affected by this circumstance at all, and therefore no striking differences would have been observed in the traditional TDM between NEO and EON. It is known that there exists no correlation between the amount of the oral dose and the steady-state trough concentration (Grevel, 1986). The only way to avoid early rejection, because of low exposure to CsA, is to use the C₂ or C₃ methods, AUC monitoring, or limited sampling strategies (e.g., absorption profiling) (Dunn et al., 2001) as input for the TDM, but these are more difficult to implement in the daily clinical routine as compared with measuring the C₀ levels, since the time window at e.g., 2 h, where the blood sample would have to be drawn, is much smaller (approximately 10 min) than by using C₀ levels. Another problem of CsA therapy is the malabsorbers. It has been suggested that specific CM measurements in the urine can be used to identify these patients as early as possible to avoid rejection episodes (Christians et al., 1991). We found elevated AM4N levels in the blood, kidney, and urine combined with an incomplete and delayed CsA absorption in the EON group. But we neither know if CsA malabsorption (e.g., due to genetic disposition) always leads to high AM4N levels, nor how much the CM profile is generally influenced in the clinical situation, where drug interactions (e.g., statins, antibiotics, diuretics) must be considered as well. Further studies with human volunteers compared with renal-transplanted patients are necessary to elucidate these connections. As mentioned in the introduction, it remains unclear if elevated levels of AM4N contribute to nephrotoxicity or if they have useful immunosuppressive activity, because these findings are mainly based on results obtained in vitro experiments (Radeke et al., 1992). In cell culture, AM4N showed changes consistent with vacuolization seen in tubular cells exposed to CsA in vivo, whereas other CM that circulate in higher concentrations (e.g., AM1) did not cause such damages (Copeland et al., 1990). Although extrapolation to humans is not possible at this time, our data in rats suggest the need of further investigation.

### Table 2

**Pharmacokinetic parameters of CsA and CM in rats after 10mg/kg p.o. under SS and SD conditions obtained in the kidneys.**

Values are means with SD in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Tₘ₉₉</th>
<th>Cₘ₉₉</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h</td>
<td>µg/ml</td>
<td>mg·h/l</td>
</tr>
<tr>
<td>Kidney SS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CsA</td>
<td>2</td>
<td>4</td>
<td>11.9 (1.79) 7.9 (1.25) 220.8 (29.5) 136.2 (21.2)</td>
</tr>
<tr>
<td>AM1</td>
<td>2</td>
<td>8</td>
<td>0.79 (0.1) 1.43 (0.22) 20.5 (3.56) 54.3 (11.2)</td>
</tr>
<tr>
<td>AM4N</td>
<td>8</td>
<td>8</td>
<td>0.57 (0.09) 0.59 (0.11) 11.8 (1.87) 12.1 (2.14)</td>
</tr>
<tr>
<td>AM9</td>
<td>8</td>
<td>8</td>
<td>0.81 (0.14) 1.12 (0.18) 23.1 (3.11) 26.3 (4.23)</td>
</tr>
<tr>
<td>Kidney SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CsA</td>
<td>2</td>
<td>4</td>
<td>8.1 (0.97) 5.9 (1.06) 103.4 (16.2) 61.5 (12.3)</td>
</tr>
<tr>
<td>AM1</td>
<td>8</td>
<td>4</td>
<td>0.85 (0.23) 0.83 (0.13) 17.5 (3.56) 21.4 (4.07)</td>
</tr>
<tr>
<td>AM4N</td>
<td>4</td>
<td>4</td>
<td>0.19 (0.06) 0.28 (0.07) 2.3 (0.51) 3.1 (0.8)</td>
</tr>
<tr>
<td>AM9</td>
<td>8</td>
<td>8</td>
<td>0.61 (0.11) 0.78 (0.14) 15.2 (2.08) 17.6 (3.12)</td>
</tr>
</tbody>
</table>

* p < 0.05 compared to kidney NEO SS.

* * p < 0.05 compared to kidney NEO SD.

![Fig. 3](image-url)
References


