EFFECTS OF TAUROURSODEOXYCHOLATE SOLUTIONS ON CYCLOSPORIN A BIOAVAILABILITY IN RATS

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ABSTRACT:

Cyclosporin A (CsA) exhibits poor bioavailability after oral administration of Sandimmune, with wide intra- and interindividual variations. Our study sought to determine the effect of the coadministration of CsA standard oily formulation and tauroursodeoxycholate (TUDC) and that of an aqueous micellar solution containing TUDC, monoolein, and CsA in promoting and regulating CsA bioavailability in the rat. Pharmacokinetic parameters of CsA were determined in fasted rats after either an intravenous administration (5 mg/kg) or a single oral CsA dose of 10 mg/kg. Compared with oral Sandimmune, the CsA micellar solution significantly improved the CsA bioavailability by 15–160% and decreased the interindividual variability in bioavailability expressed as percent coefficient of variation from 32% to 15%. The concentration-time profile was modified with a 3.5-fold increase in Cmax, a reduction of tmax, and an increased trough concentration. Bioavailability slightly improved in rats receiving standard oily solution plus concomitant TUDC, although not significantly. Data indicate that the structure of the CsA carriers greatly affect drug bioavailability and that aqueous micellar solutions of CsA-TUDC-monoolein constitute efficient vehicles, thus providing for CsA high absorption with low variability.

CsA is a potent immunosuppressive drug widely used in organ transplantation. CsA exhibits a low therapeutic index and a poor bioavailability with strong intra- and interindividual variations. In humans, the bioavailability of CsA has been reported to range from 10 to 50%, with a mean of ~30% (1, 2) and from 12 to 23% in rats (3). These pharmacokinetic parameters impede the use of CsA and necessitate close monitoring to ensure adequate blood concentrations.

A major factor of this poor bioavailability is the low absorption of CsA from the vegetable oil- and ethanol-containing Sandimmune (4). Furthermore, food intake, bowel length, pancreatic exocrine secretion, and bile flow are largely known to influence CsA absorption (5–9). Thus, as for other fat-soluble substances, CsA absorption is adversely affected by biliary diversion and cholestasis, as shown in animals (10, 11) and in liver transplant patients (2, 6, 12). With regard to the CsA poor aqueous solubility, a carrier is necessary to allow CsA to migrate into the enterocyte. Taken together, these considerations suggest that the drug delivery system is bile salt micelle-mediated diffusion.

UDC is a natural bile salt only present in small quantities in humans. This bile salt is very hydrophilic and in this sense is distinct from other human bile salts (13). UDC is devoid of any toxicity, and it has been shown to be an effective treatment for various cholestatic liver diseases (14–17). In recent studies (18, 19), we have demonstrated the protective role of TUDC, a conjugated form of UDC, on hepatic disorders induced by CsA, especially cholestasis. These considerations prompted us to investigate the pharmacokinetics of CsA with or without TUDC in rats. Our objective was to study the effectiveness of the coadministration of TUDC and that of an aqueous TUDC-monoolein-CsA micellar solution in maximizing and regulating CsA bioavailability.

Materials and Methods

Chemicals. Concentrated solution (Sandimmune) for intravenous perfusion, oral solution (Sandimmune), CsA powder, and Sandimmune placebo were kindly provided by Sandoz Ltd. (Basel, Switzerland). Intravenous solution contained 50 mg/ml of CsA, polyoxyethylated castor oil (Cremophor EL) and ethanol. Oral solution contained 100 mg/ml of CsA dissolved in a mixture of olive oil, pegylol 5-olate (Labrafil), and ethanol. Sodium TUDC (>99% pure) was purchased from Calbiochem (Los Angeles, CA). 1-Monoolein (99% pure) was from Sigma Chemical Co. (St. Louis, MO).

Animals. Thirty male Sprague-Dawley rats weighing 220–240 g were obtained from Ifa-Credo (l’Arbresle, France). They were housed under normal laboratory conditions and had free access to chow and water. Animals received humane care, and studies were approved by the Service Vétérinaire de la Santé et de la Protection Animale (no. 001456).

Experimental Design. Rats were anesthetized with pentobarbital (50 mg/kg ip) to perform a cannulation of the femoral vein for blood sampling with a Veinocath 18 catheter; an additional jugular vein catheter for infusion of the drug was inserted into rats receiving intravenous CsA. Surgery was performed 12 hr before CsA administration to avoid hepatic metabolic interference. All animals were then housed in restraining wire-bottom cages to avoid contact with fecal excrement. The rats remained fasting for a 12-hr period before CsA administration and for a further 6 hr thereafter. Water was freely available throughout the experimentation. Because CsA pharmacokinetics and toxicity are time-dependent, single doses of drug were administered at about the same time (between 8 and 10 a.m.). An intravenous infusion of 0.9% NaCl was maintained at a rate of 1 ml/hr by means of an infusion pump throughout the experiment. The rats were randomly distributed into four groups and received a single dose of CsA as follows:

1 Abbreviations used are: CsA, cyclosporin A; UDC, ursodeoxycholate; TUDC, tauroursodeoxycholate; AUC, area under the blood drug concentration-time curve; CV, coefficient of variation.
• CsA intravenous group (group I)—For intravenous perfusion, Sandimmune for injection was diluted extemporaneously with normal saline to obtain a concentration of 2.5 mg/ml. A single dose (5 mg/kg) was given for about 1 min to seven rats.

For oral administration, Sandimmune oral solution was diluted 20-fold with Sandimmune placebo and then given as follows:

• CsA group (group II)—A single dose of CsA (10 mg/kg) was administered by gavage (volume ~0.5 ml) to eight rats, immediately followed by the administration of 0.5 ml of 0.9% NaCl.

• CsA + TUDC group (group III)—CsA (10 mg/kg) and a single dose of TUDC (200 mg/kg) dissolved in 0.9% NaCl were coadministered by gavage (total volume of 1 ml) to seven rats.

• CsA-containing micelles group (group IV)—A single dose of CsA powder (10 mg/kg) solubilized in a micellar solution of TUDC (100 mM) and monoolein (60 mM) was administered by gavage (volume ~1 ml) to eight rats.

Blood samples of 100 μl each were collected from the precannulated femoral vein into 2-ml EDTA tubes. The sampling times were: 1, 2, 3, 3.5, 4, 4.5, 5, 7, 12, 24, and 30 hr postoral administration; and 5, 10, and 30 min and 1, 2, 3, 3.5, 4, 4.5, 5, 7, 12, and 24 hr for intravenous-administered rats.

Blood Level Monitoring. CsA blood levels were measured in duplicate using a specific monoclonal radioimmunoassay (CYCLO-Trac, Sorin-France S.A. Antony, France). The kit uses a specific monoclonal antibody that measures only cyclosporin and demonstrates no significant cross-reactivity with its metabolites. Average cross-reactivity of CsA metabolites is calculated as <0.1–1.7% for the various major metabolites. The within-assay variation precision is 3.2% at 186 ng/ml and up to 10.7% at 46 ng/ml CsA concentration. Between-assay variation at 200 ng/ml is ~7%. The sensitivity of the method defined at three standard deviations from the count at maximum binding is 8.7 ng/ml.

Micellar Solubilization of CsA. The micellar solubilization of CsA in TUDC/monoolein solutions was determined using the coprecipitation method (20). Increasing amounts of CsA were added to mixtures with a fixed molar ratio of bile salt to monoolein of 1.67. The desired lipid concentration (100 mM TUDC and 60 mM monoolein) was obtained by adding aqueous solvent (0.15 M NaCl; pH 6.5). Samples were then flushed with purified N2, sealed, and equilibrated at 30°C for 5 days. Separation of a second phase from the isotropic solutions was monitored by optical methods: photon microscopy and scattered light (Tyndall phenomenon).

Data Analysis. The APIS computer program for clinical pharmacokinetics (APIS Software, Mipps, Marseille, France) was used to determine the pharmacokinetic parameters. All concentration-time profiles were plotted linearly and evaluated individually. The AUC from dosed-corrected AUC after a single oral dose to the dose-corrected AUC of CsA intravenous group (group IV) was strongly increased, compared with CsA standard formulation (group II) (p = 0.0012) and group III (p = 0.012). Conversely, time of maximum blood drug concentration was not significantly different between groups II and III.

Maximum whole blood drug concentration was significantly higher in group IV than in groups II and III (p = 0.0012 and p = 0.0045, respectively), and in group III than in group II (p = 0.0279).

The absolute bioavailability of CsA administered in micellar solution (group IV) was strongly increased, compared with CsA standard formulation (group II) (p = 0.0012) or with CsA standard formulation plus concomitant TUDC (group III) (p = 0.0045). Bioavailability from oily CsA plus concomitant TUDC was increased, although not significantly, when compared with standard formulation.

CV: The variability of bioavailability in group IV was 2-fold lower when compared with group II (CV = 15% vs. 32%). The variances of these groups did not differ significantly. So, the differences in CV we found were caused by differences in means. However, when TUDC was simply added as a concomitant application to oily CsA, bioavailability values were similarly affected by large interindividual CV.

Discussion

This work was designed to compare the CsA bioavailability from oral oily or aqueous micellar solutions of CsA. Our findings demonstrate that the CsA micellar solution significantly improves CsA bioavailability and decreases the interindividual variability in bioavailability.

The increase of CsA bioavailability induced by TUDC micellar solution could be due to a decrease of the CsA biliary excretion. In fact, it has been previously shown (21) that a competitive inhibition of ATP-dependent bile salt transport by CsA in the canalicular membrane strongly reduced bile flow and, on the other hand, that TUDC was able to counteract cholestasis and to increase the biliary excretion of endogenous bile salts and CsA (18, 19). Thus, the hypothesis of a decreased CsA elimination by TUDC can be ruled out, and the improvement of CsA bioavailability seems to be essentially the consequence of the increased CsA intestinal absorption.

In physiological conditions, lipids are absorbed mainly from the micellar phase, with this phase being continuously generated from the emulsified oil phase. Efficient fat absorption necessitates the rapid flux of insoluble molecules through the very thick unstirred water layer coating the absorptive mucosa of the small intestine (22). Intes-
tinal mixed micelles have a very small particle size with large translational self-diffusion coefficients (23) and thus are able to overcome efficiently the resistance of the unstirred layer.

CsA is a highly lipophilic cyclic nondecapeptide whose solubilization in the intestinal lumen strongly depends on lipids. Absorption of CsA is well described by first-order kinetics (24), suggesting that CsA penetrates into the enterocytes by passive diffusion. Consequently, the nature of lipids dissolving CsA and the structure of the CsA carriers

Fig. 1. Individual blood CsA concentrations versus time after single oral administration of 10 mg/kg CsA in rats.

(A) Group II: CsA oily formulation. (B) Group III: Oral coadministration of CsA oily formulation + TUDC. (C) Group IV: TUDC-monoolein-CsA micellar solution.
TABLE 1
Pharmacokinetic parameters obtained from the analysis of blood concentration after a bolus intravenous dose of 5 mg/kg of CsA and after three oral administrations of 10 mg/kg of CsA

<table>
<thead>
<tr>
<th>Group</th>
<th>AUC (µg hr/ml)</th>
<th>t_{1/2} (hr)</th>
<th>t_{max} (hr)</th>
<th>C_{max} (µg/ml)</th>
<th>F (%)</th>
<th>CV of F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CsA Intravenous Administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>48.63 ± 7.64</td>
<td>12.83 ± 1.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CsA Oral Administration</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>II</td>
<td>15.02 ± 4.82</td>
<td>11.79 ± 2.56</td>
<td>5.47 ± 1.19</td>
<td>0.88 ± 0.30</td>
<td>15.5</td>
<td>± 4.9</td>
</tr>
<tr>
<td>III</td>
<td>19.85 ± 5.27</td>
<td>12.85 ± 2.28</td>
<td>4.43 ± 1.62</td>
<td>1.32 ± 0.35</td>
<td>20.4</td>
<td>± 5.4</td>
</tr>
<tr>
<td>IV</td>
<td>39.95 ± 5.90</td>
<td>11.47 ± 1.87</td>
<td>1.81 ± 0.75</td>
<td>2.73 ± 0.48</td>
<td>41.5</td>
<td>± 6.1</td>
</tr>
</tbody>
</table>

Group I (N = 7): CsA intravenous; group II (N = 8): CsA oily solution; group III (N = 7): CsA oily solution + concomitant TUDC; group IV (N = 8): TUDC-monoolein-CsA micellar solution. Values are expressed as means ± SD. t_{1/2}, half-life; t_{max}, time to maximum concentration; C_{max}, maximum concentration; F, bioavailability.

- Significantly different from CsA oily solution (p < 0.05).
- Significantly different from CsA oily solution + concomitant TUDC (p < 0.05).
- Significantly different from TUDC-monoolein-CsA micellar solution (p < 0.05).

(i.e. emulsion, microemulsion, and micelles) have the potential to alter drug absorption (25).

The present data show that TUDC-monoolein mixed micelles are able to solubilize CsA and to enlarge the CsA intestinal absorption. These micellar aggregates increase the CsA aqueous solubility up to 2 mM, a value ~400-fold larger than that of CsA in pure water. These findings confirm the major role of bile salts in CsA absorption as observed in clinical practice by the increased absorption of CsA from the oily formulation after restoration of biliary function by T-tube clamping in liver-transplanted patients (6, 12). Similarly, the absorption of CsA from the microemulsion formulation, Neoral, is not completely independent of bile flow. An inverse correlation was observed between the volume of externally drained bile and the bioavailability of CsA (26). However, oral bile acid supplementation in safe volunteers (7) or in liver transplant recipients (27) resulted in only a limited enhancement of CsA absorption. These features are in agreement with our results, as expressed by the rather similar absorption parameters of groups receiving the oily formulation and the oily formulation plus TUDC. In fact, bile acids poorly mix with CsA and are not sufficient by themselves to improve CsA absorption significantly. A tight molecular association of the micellar type seems necessary to ensure a large passage of CsA into the mucosa. The present study demonstrates the usefulness of a micellar association between CsA, TUDC, and monoolein to control and improve CsA absorption. Mixed micelles of TUDC-monoolein act as rapid carriers of CsA, due to their small particle size (28). The effect of this micellar solubilization is to accelerate diffusion through the unstirred layer coating the intestinal mucosa and to carry efficiently the drug into enterocytes. On the other hand, TUDC is known to be absorbed exclusively in the ileum under action of specific receptors. Thus, TUDC is available on the major part of the small intestine to facilitate CsA carriage. Lastly, the micellar solution of CsA resulted in less variability of bioavailability than the oily formulation. CsA-containing micelles permit CsA to break away from dependence on digestive secretions and to present to the mucosa the ultimate form of lipid digestion from which absorption mainly takes place.

To our knowledge, the use of a micellar association between CsA, TUDC, and monoolein has never been reported before. Our experimental results in rats are promising and should lead to studies in humans, with adapted TUDC dosage, because CsA bioavailability is of some predictive value for the incidence of graft survival and rejection (29). In transplanted patients (e.g. liver recipients), intra- and interindividual variations in CsA pharmacokinetics are frequent. Sometimes, massive dosage of CsA is required to obtain therapeutic blood concentrations, as in cystic fibrosis patients or in patients with reduced intestinal absorptive area (8, 30, 31). In our study, it is noteworthy that larger CsA blood concentrations with the mixed micellar solution were maintained throughout the first 30 hr, when compared with the other oral forms. Thus, an increase in CsA bioavailability may favor the use of a lower dosage of the drug without altering the therapeutic effect.

In addition to the beneficial effect of TUDC on CsA bioavailability, TUDC administration improves CsA-induced cholestasis. This has been shown in the rat, in either long-term or acute administration (18, 19). Similarly, in humans, one study (32) has reported the disappearance of cholestasis under UDC in heart-transplanted patients. Two further findings are of interest. First, a TUDC positive effect on bile secretion was associated with an increased excretion in bile of the CsA parent molecule and its metabolites. Second, the CsA/ CsA + metabolites ratio was maintained in blood under TUDC (19). Thus, on the whole, TUDC appears to improve the CsA intestinal absorption and diminish CsA-induced cholestasis.

In conclusion, our data show that, in the rat model, an aqueous mixed micellar solution containing TUDC and monoolein increases CsA bioavailability as a result of improved CsA absorption. These results need confirmation using long-term treatment protocols and further studies in humans.

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References


