Cyclosporin A (CsA) is currently the main immunosuppressant used in solid organ transplantation. This compound binds to a cytoplasmic cyclophilin, and the resulting complex inhibits calcineurin and, hence, interleukin 2, which blocks transcriptional activation of T cells (Graham, 1994). This immunosuppressant initially was used in the early 1980s. Recent research has been conducted into both improving the formulation of CsA delivery and developing other compounds to improve immunosuppression either as replacements for, or to work in synergy with, CsA (Kahan et al., 1991; Lake and Canafax, 1995; Lampen et al., 1995).

Allograft rejection in organ transplantation is an area that should be reduced greatly with the advent of the new, orally active immunosuppressant, SDZ-RAD. This compound is a derivative of rapamycin, which inhibits the proliferation of T cells by a different mechanism than that of CsA, preventing their entry into the S phase of cell division (Goral and Helderman, 1997). However, animal and human studies have shown that the absorption and bioavailability of rapamycin have considerable variability (Granger et al., 1995; Ferron et al., 1997).

One of the significant contributions to intestinal uptake variability is active efflux back to the luminal by P-glycoprotein (P-gp) and other active efflux pumps present in the apical layer of enterocytes at the villus tip of intestinal microvilli. P-gp originally was identified as an efflux pump responsible for multidrug resistance in tumor cells, but since has been found to be expressed in many tissues of normal cells including the kidney, blood-brain barrier, and enterocytes of the intestine (Cordon-Cardo et al., 1989; Augustijns et al., 1993; Hunter et al., 1993). Recent reports suggest that rapamycin is a substrate for P-gp, as are many immunosuppressive compounds including CsA and tacrolimus (Augustijns et al., 1993; Hoof et al., 1993; Hebert, 1997). Current results from our own group suggest that SDZ-RAD also is a substrate for P-gp (Crowe and Lemaire, 1998). With the confirmed presence of P-gp in the intestine and its links with metabolizing enzymes of the CYP3A class (Gan et al., 1996), emphasis is being focused on the relevance of intestinal metabolism when determining the effect of first-pass metabolism on these compounds and, hence, their bioavailability. Other in vitro and in situ studies in our laboratory indicated that SDZ-RAD has better intestinal absorption than its parent compound, rapamycin (Crowe and Lemaire, 1998). This study aims to expand on our early in vitro and in situ observations by examining the difference in absorption and disposition between SDZ-RAD and rapamycin in a rat model focusing on metabolism at the intestine. Potential synergistic action between SDZ-RAD and CsA also was explored to determine whether these two P-gp substrates could increase each other’s intestinal absorption.

Materials and Methods

Materials. [3H]SDZ-RAD (44.67 MBq/μmol) and [14C]rapamycin (1.571 MBq/μmol) were prepared by Novartis’ isotope laboratory and shown to be higher than 99% pure by HPLC analysis. Placebo microemulsion (as described in Schuler et al., 1997), concentrate for infusion (polyethoxylated castor oil and ethanol 65:35, w/w), nonlabeled SDZ-RAD, nonlabeled rapamycin, and CsA also were prepared from within Novartis.
Animal Studies. The experiments were performed with male Wistar rats weighing approximately 300 g (BRL, Fuellinsdorf, Switzerland). They were anesthetized with Metofane (methoxyflurane; Mallinckrodt Veterinary Inc., Mundelein, IL) in a veterinary respirator (model HH64; Holzel). The right femoral artery was cannulated with a segment of polyethylene tubing containing heparinized saline (100 U/ml) for the collection of blood. Rats that were infused i.v. also had the right femoral vein cannulated. The tubes were passed s.c. to emerge at the base of the neck. Animals were isolated in metabolic cages and allowed to move freely. Full recovery from anesthesia usually occurred within 2 h, and the presence of the catheter(s) caused no obvious discomfort to the animals. Administration of the drugs was carried out the following day, after surgery. Animals had access to food before and after surgery, but this was removed 14 h before administration of the compounds. All animals had free access to drinking water.

Dosage and Administration. Intravenous infusion. [3H]SDZ-RAD was mixed with nonlabeled SDZ-RAD to obtain the appropriate specific radioactivity, whereas [14C]rapamycin was used without nonlabeled additions. Both compounds were dissolved in microemulsion and diluted in saline (1:2.6, v/v). The formulations were administered as a 2-h infusion (0.3 ml/h) in the cannulated femoral vein. The dose for both compounds was 1 mg/kg. Blood was collected from the cannulated femoral artery 1 h before the end of infusion, at the end of infusion (time 0), and at 0.5, 1.0, 2.0, 4.0, 8.0, 24, 32, 48, 72, and 168 h after drug infusion had ceased.

Oral administration. [3H]SDZ-RAD and [14C]rapamycin were dissolved in microemulsion and diluted to a final volume with saline. The dose ingested was 1.5 mg/kg using 5.0 ml/kg of the saline/microemulsion mixture administered as a 2-h infusion (0.3 ml/h) in the cannulated femoral vein. The dose for both compounds was 1 mg/kg. Blood was collected from the cannulated femoral artery 1 h before the end of infusion, at the end of infusion (time 0), and at 0.5, 1.0, 2.0, 4.0, 8.0, 24, 32, and 48 h after gastric intestinal rat dose was then added to the mixed blood, and the resulting samples were pipetted into the precoated wells and sealed at 4°C for 150 min. After streptavidin-peroxidase and peroxidase substrate additions, the microtiter plates were examined using Student’s two-tailed unpaired or paired t test as appropriate. Results were considered significant if P < .05.

Results

The mean blood concentrations of both SDZ-RAD and rapamycin after i.v. and oral administration can be seen in Fig. 1. Blood concentrations of intact SDZ-RAD from a 2-h infusion of 1 mg/kg SDZ-RAD were shown to be cleared more rapidly than that of an equivalent dose of rapamycin (Fig. 1A). When the AUCs were calculated to infinity (Table 1), it was established that rapamycin had an AUC which was double that of SDZ-RAD (1140 compared with 573 ng*h/ml). However, when applied via the oral route, blood concentrations of intact SDZ-RAD and rapamycin were very similar. A comparison of blood levels obtained after i.v. and oral administration indicated a low absorption of both compounds; however, the absorption that did occur proceeded very rapidly—the highest blood concentrations were observed after only 30 min (Fig. 1B). A clear biphasic response in elimination of orally presented rapamycin was observed in this study, which was much more pronounced than that of SDZ-RAD (Fig. 1B). Up to 12 h of elimination of rapamycin was quite rapid whereas elimination after 12 h was significantly lower. The first phase would correspond to rapid tissue distribution whereas the second was most likely limited by systemic metabolism (Fig. 1A). Blood samples from the oral dose rapamycin experiment was collected only for 48 h; therefore, for consistency, all results in Table 1 were calculated from the extrapolation of 0- to 48-h data. It can be seen that very little difference existed in the primary half-life of rapamycin and SDZ-RAD regardless of the route of administration. However, the terminal half-life of rapamycin was 25 h using the 0- to 48-h data compared with only 15 h for SDZ-RAD (Table 1). Again, no difference in half-life was apparent between oral and i.v. doses. The systemic bioavailability of SDZ-RAD, estimated by the ratio of dose-normalized blood AUCs, amounted to more than 16% whereas the bioavailability of rapamycin was only 10% in comparison.
Results are mean ± SEM of 4-5 rats each.

Fig. 1. Mean blood concentration in adult male rats of rapamycin and SDZ-RAD after a 2-h infusion of either 1 mg/kg rapamycin or SDZ-RAD (A; inset shows a magnification of blood concentrations over the first 8 h) and an oral gavage injection of 1.5 mg/kg rapamycin or SDZ-RAD (B).

Results are the mean ± S.E.M. of four rats.
Table 1

Pharmacokinetic parameters for SDZ-RAD and rapamycin in adult rats after single oral administrations (1.5 mg/kg) and 2-h i.v. infusions (1.0 mg/kg)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route</th>
<th>$AUC_{0-\infty}$</th>
<th>$AUC_{0-\infty}$</th>
<th>CL</th>
<th>$V_{ss}$</th>
<th>$T_{1/2}$</th>
<th>$T_{1/2}$</th>
<th>MRT</th>
<th>F</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ng·h·ml⁻¹</td>
<td>ng·h·ml⁻¹</td>
<td>ml/min</td>
<td>kg</td>
<td>h</td>
<td>h</td>
<td>h</td>
<td>%</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>Oral</td>
<td>428 ± 12</td>
<td>172 ± 10</td>
<td>24 ± 0.5</td>
<td>2.4 ± 0.5</td>
<td>24.3 ± 4.6</td>
<td>23.8 ± 5.3</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>SDZ-RAD</td>
<td>Oral</td>
<td>606 ± 36</td>
<td>141 ± 43</td>
<td>2.7 ± 0.5</td>
<td>1.9 ± 0.1</td>
<td>25.3 ± 2.6</td>
<td>34.5 ± 4.2</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>Rapamycin</td>
<td>i.v.</td>
<td>1760 ± 30</td>
<td>1140 ± 90</td>
<td>3.0 ± 0.2</td>
<td>27 ± 3</td>
<td>25.2 ± 2.6</td>
<td>34.5 ± 4.2</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>SDZ-RAD</td>
<td>i.v.</td>
<td>1350 ± 90</td>
<td>573 ± 32</td>
<td>52 ± 4</td>
<td>3.4 ± 1.1</td>
<td>14.2 ± 0.4</td>
<td>15.9 ± 0.5</td>
<td>16.4</td>
<td></td>
</tr>
</tbody>
</table>

$T_{1/2}$ is half-life of main elimination phase, and $T_{1/2}$ is half-life of the terminal phase based on the 0- to 48-h data only. Results are expressed as means ± S.E.M. of four to five rats.

SDZ-RAD is a structural analog of rapamycin, sharing the same mechanisms of action (Schuler et al., 1997). It was of interest to determine the oral absorption profile of the new immunosuppressant SDZ-RAD and compare it with rapamycin because a previous report by Schuler et al. (1997) reported that even though the in vitro activity of SDZ-RAD was less than half that of rapamycin, in their rat model, oral activity was equivalent for the two compounds.

The results of this study showed that when both compounds were given i.v., rapamycin had a much longer residence time in the blood compared with SDZ-RAD. However, it was shown clearly that when both compounds were administered orally, the blood AUCs for SDZ-RAD and rapamycin were similar, consistent with the oral equipotency of these two immunosuppressants described recently (Schuler et al., 1997). Hence, the bioavailability of SDZ-RAD was 60% higher than that of rapamycin, most likely because of more rapid absorption of SDZ-RAD across the intestinal wall. This study used normal, healthy rats, but a recent study has examined the pharmacokinetics of rapamycin in human kidney transplant patients and concluded that the oral absorption was 20% (Ferron et al., 1997), about double our estimate in rats from this study. However, the variability seen in their study suggests that interpatient differences in rapamycin bioavailability could range from 3 to 35%, just as CsA is reported to have a
The first 12 h of oral absorption showed rapamycin to have a slightly faster rate of blood drug level decrease than SDZ-RAD. The opposite effect was observed after i.v. administration, which could indicate that gastrointestinal first-pass metabolism was the initial limiting factor in circulating blood levels of rapamycin, but that once most of the compound had been absorbed, then systemic clearance became the limiting factor.

Of the current immunosuppressants, P-gp’s role in CsA activity and absorption has been examined by many studies (Zacher et al., 1994; Fricker et al., 1996; Tanaka et al., 1996; Terao et al., 1996). It was found that P-gp efflux at the intestinal wall accounts for almost half of the variability in bioavailability of CsA (Lown et al., 1997). After oral application in this study, the percentage of parent compound remaining in the blood was lower for both SDZ-RAD and rapamycin in comparison to i.v. dosing, suggesting that intestinal metabolism was occurring, and this was confirmed from analysis of mesenteric vein blood immediately after absorption. Therefore, we also would conclude from our animal studies that significant proportions of both rapamycin and SDZ-RAD, especially at low oral doses, are affected by intestinal first-pass metabolism and that P-gp efflux may be a factor in this effect along with CYP3A4 and other cytochrome P-450s.

The mesenteric vein study showed that SDZ-RAD was metabolized by the intestine to a greater extent than rapamycin. SDZ-RAD also was eliminated from the blood faster than rapamycin after i.v. administration because of the combined effect of increased clearance and a much higher volume of distribution. However, the AUCs after oral application were similar for both compounds, which suggests that SDZ-RAD has a very high rate of absorption to counteract its metabolism and elimination pathways. This conclusion is supported by other recent results in our laboratory that showed SDZ-RAD to have almost double the intrinsic permeability of rapamycin through our in vitro Caco-2 intestinal transport system (Crowe and Lemaire, 1998). It is interesting that SDZ-RAD differs from rapamycin solely by the addition of a 40-O-(2-hydroxymethyl) group. This alteration appears to be enough to completely alter membrane absorption, metabolic enzyme affinity, and pharmacokinetic profiles, as suggested here and elsewhere (Schuler et al. 1997; Crowe and Lemaire, 1998), yet with these differences, systemic exposure after oral dosing is similar for rapamycin and SDZ-RAD. Because absorption is rapid for SDZ-RAD, it may be possible to inhibit intestinal metabolism by allowing greater amounts of the compound to enter the circulation intact.

It has been shown that the addition of SDZ-RAD in conjunction with the P-gp and CYP3A inhibitor, CsA, can result in an increase in blood levels of CsA, just as CsA can equally increase blood levels of SDZ-RAD. A recent publication examined the blood concentrations of CsA and rapamycin after i.v. coadministration in rats, and rapamycin was able to increase CsA blood concentrations but CsA was not
able to increase rapamycin levels, even though it was present at 50 times the concentration of rapamycin (Stepkowski et al., 1997). However, a recent peroral study in rats examining rapamycin tissue and blood levels after a 14-day coadministration with CsA did show increased blood rapamycin levels after 14 days when CsA was coadministered at 6.5 times the concentration of rapamycin (Napolitano et al., 1998). One of their dosages (2.5:0.4 mg/kg, CsA:rapamycin) was very similar to ours (2.5:0.6 mg/kg, CsA/SDZ-RAD), so it was interesting to see that this dosage of CsA could double mean rapamycin blood levels after 14 days, whereas our results show this concentration of CsA increased the SDZ-RAD AUC by 5-fold and the maximum blood concentration by 4.5-fold. Even though their study was a sustained-exposure protocol for 2 weeks and ours was a short-term, 24-h pharmacokinetic study, it clearly can be seen that coadministration of these immunosuppressants does lead to higher concentrations in the blood after oral application. Ferron and coworkers (1997) also examined whether CsA blood concentrations were changed after giving steady-state doses to patients followed by a single dose of rapamycin, but no alterations in CsA blood levels were noted. Other groups have examined the synergistic activity of rapamycin with either tacrolimus (Arcelli et al., 1992) or CsA (Kahan et al., 1991) together, showing that combinations of immunosuppressants have an additive effect on intracellular accumulation of target drugs and increase survival of allograft transplanted animals at lower levels than could be effective singularly. Apart from showing that CsA can increase blood concentrations of SDZ-RAD, our combined results with SDZ-RAD and CsA add weight to these previous reports and suggest encouragement for synergistic action in immunosuppression therapy (Schuurman et al., 1997).

In conclusion, it has been shown that SDZ-RAD has a better oral absorption profile than rapamycin in this study, which counteracts its high rate of intestinal metabolism and systemic elimination so that both rapamycin and SDZ-RAD have similar blood levels after oral application. Also, that CsA was able to increase blood levels of SDZ-RAD shows that the additive effect of these two compounds can benefit the pharmacokinetics of both compounds.

**References**


