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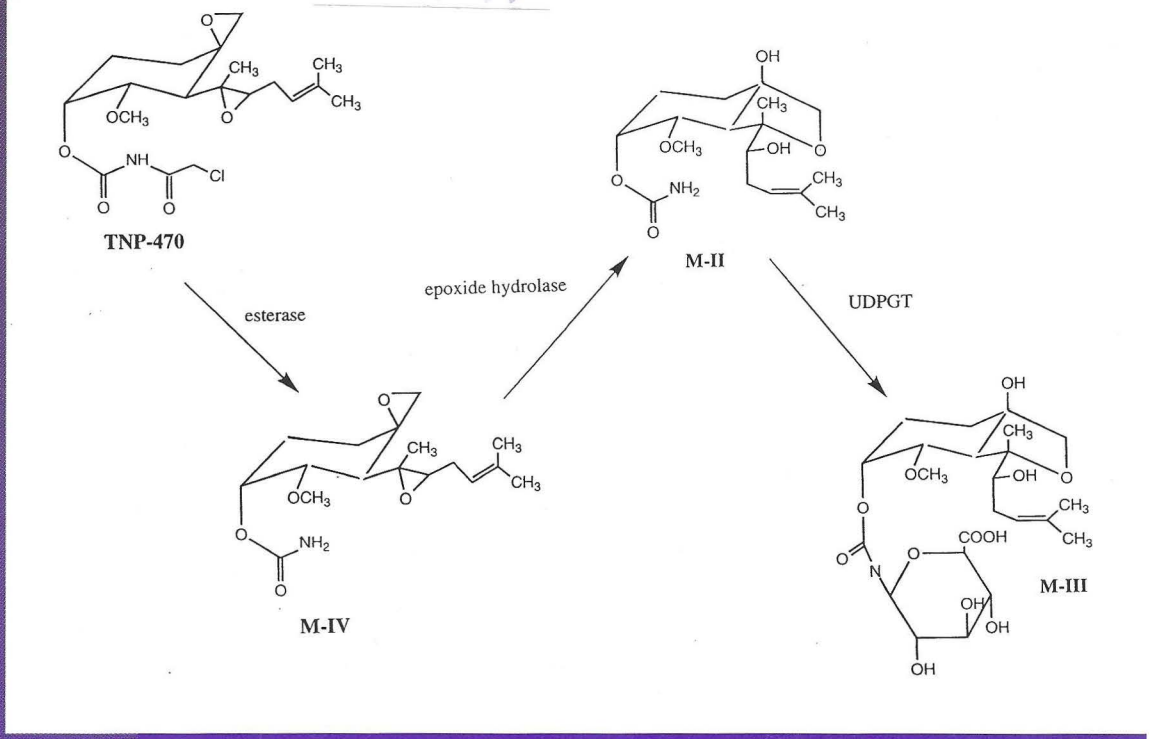
DRUG METABOLISM AND DISPOSITION



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DRUG METABOLISM AND DISPOSITION
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ABSORPTION AND INTESTINAL METABOLISM OF SDZ-RAD AND RAPAMYCIN IN RATS

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ABSTRACT:

The new immunosuppressive agent, SDZ-RAD, and its analog rapamycin were examined for intestinal absorption, metabolism, and bioavailability in Wistar rats. Intestinal first-pass metabolism studies from rat jejunum showed that at 0.5 mg of SDZ-RAD/kg rat, 50% of the parent compound was metabolized in the intestinal mucosa, and this decreased to around 30% when SDZ-RAD was increased to 5.0 mg/kg rat. Results for rapamycin at the low dose were similar to those for SDZ-RAD, but at the higher dose only 1 to 14% of the total rapamycin absorbed was metabolized by the intestine. After i.v. administration of 1 mg/kg SDZ-RAD or rapamycin, the area under the concentration curve (AUC) for rapamycin was twice that of SDZ-RAD, resulting in a systemic clearance of 6.2 ml/min and 3.0 ml/min for SDZ-RAD and rapamycin, respectively. However, the AUC for oral absorption was similar for the two compounds: 140 and 172 ng*h/ml for SDZ-RAD and rapamycin, respectively. Because blood clearance was faster for SDZ-RAD after i.v. administration, the absolute oral bioavailability for SDZ-RAD was 16% compared with 10% for rapamycin. Overall, the data suggest that intestinal first pass is a major site of metabolism for SDZ-RAD and rapamycin and that intestinal absorption of SDZ-RAD was much faster than that of rapamycin. This allowed it to counteract the combined actions of faster systemic clearance and increased intestinal metabolism, resulting in comparable absolute exposure when given orally. Also, the coadministration of cyclosporin A with SDZ-RAD was shown to dramatically increase blood AUCs for SDZ-RAD, probably through saturating intestinal metabolism mechanisms.

Cyclosporin A (CsA)[1] 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 lumen by P-glycoprotein (P-gp) and other active efflux proteins 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.

[1] Abbreviations used are: CsA, cyclosporin A; P-gp, P-glycoprotein; AUC, area under the concentration curve; LC-RID, liquid chromatography-reverse isotope dilution; AUMC, area under the mean concentration curve.

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Materials and Methods

Materials. [^{3}H]SDZ-RAD (44.67 MBq/μmol) and [^{14}C]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

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 HN64; 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.* [^{3}H]SDZ-RAD was mixed with nonlabeled SDZ-RAD to obtain the appropriate specific radioactivity, whereas [^{14}C]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. [^{3}H]SDZ-RAD and [^{14}C]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 by gastric intubation according to the individual body weight on the day of application. Blood was collected from the cannulated femoral artery 0.5, 1.0, 2.0, 4.0, 8.0, 12, 24, 32, and 48 h after gastric intubation of radiolabeled compounds. At various time points for both oral and i.v. administered rats, a volume of blood equivalent to that taken was replaced through the cannulated femoral artery using fresh blood from donor rats.

CsA, as Neoral containing 100 mg/ml CsA, was administered to rats either alone at 2.5 mg/kg by gastric intubation or in combination with 0.6 mg/kg [^{3}H]SDZ-RAD for the oral coadministration study. [^{3}H]SDZ-RAD also was used alone at 0.6 mg/kg in this study.

Mesenteric Vein Method. Adult Wistar rats (300 g) were fasted overnight before initiating the dosing study. Rats were anaesthetized for the duration of the study with i.p. injections of urethane (1.1 g/kg). The middle 10 cm of the jejunal segment was ligated in a location that allowed all blood from mesenteric branching along the ligated segment to be collected from one point just before entering the superior mesenteric vein. One milliliter of placebo microemulsion/saline mix (1:13.5), containing either 0.15 mg or 1.5 mg of a [^{3}H]SDZ-RAD solution (189–329 μCi/ml), or a [^{14}C]rapamycin solution (7.0–13.9 μCi/ml), was injected into the segment. The total mesenteric blood for the region was collected immediately using a 27-gauge needle and 1-ml syringe. Collection continued for as long as possible, usually 3 to 5 min, allowing no blood to enter the circulation from the ligated segment. Unlike other studies examining mesenteric vein blood that have used lengths of time of more than 30 min (Kim et al., 1993), we did not want to perfuse the animals with donor blood, limiting our study to the initial absorption phase (approximately 5 min). Blood of individual animals was examined for total radioactivity by liquid scintillation counting and for parent drug by liquid chromatography-reverse isotope dilution (LC-RID).

Parent Drug Determinations (LC-RID). Blood aliquots (200 μl) were spiked with 200 μl of cold compound (50 μg/ml of either SDZ-RAD or rapamycin in acetonitrile). Water (1 ml) and 100 μl of 5× concentrated Merck Titrisol, pH 9.0, buffer also were added. Two hundred fifty microliters of SDZ-RAD and rapamycin was extracted in diethylether, evaporated, and reconstituted in mobile phase consisting of acetonitrile/tertiary butyl methylether and 0.1% tetramethylammonium hydrogen sulfate (370:60:500, w/w/w). Seventy-five microliters of *n*-hexane was then added. Samples were vortexed vigorously, and, after centrifugation, the hexane layer was removed. SDZ-RAD and rapamycin in the remaining phase were separated from their metabolites by HPLC. Chromatography was performed on a HPLC system (Kontron Instruments, Zurich, Switzerland). Separation was conducted on a Brownlee Spheri-10 RP2 column (4.6 × 220 mm) at 70°C. The mobile phase was as at 278 nm, and the peak corresponding to either unchanged [^{3}H]SDZ-RAD or [^{14}C]rapamycin was collected in a polyethylene vial by fraction collection (SuperFrac; Pharmacia LKB, Uppsala, Sweden) and subjected to radioactivity determinations. The concentration of parent compound in each sample was calculated from the ratio of the amount of radioactivity in the collected fraction to the area of the UV absorbance of the nonradiolabeled SDZ-RAD or rapamycin as used as internal standards (Everett et al., 1989).

CsA Determinations (enzyme-linked immunosorbent assay). CsA was determined using the whole-blood cyclosporin displacement immunoassay procedure. Microtest plates were coated with goat anti-mouse (Fc) in coating buffer. Twenty-five microliters of whole blood was mixed with 75 μl of CsA displacement/lysis buffer. Biotinylated CsA and a CsA-specific monoclonal antibody were 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 at 490 nm in a spectrophotometric plate reader.

Data Analysis. Blood levels of unchanged SDZ-RAD and rapamycin were evaluated by nonlinear regression analysis using the noncompartmental model of constant infusion for i.v. doses and extravascular input for oral doses using the WinNonlin Pro package for Windows NT 4.0 (Scientific Consulting Inc., Cary, NC), using a 166-MHz Pentium computer. AUC and area under the mean concentration curve (AUMC) of the blood-drug concentration time curves were obtained by the linear trapezoidal rule and extrapolated to infinite time by the additions of $C(t_m)/\lambda_z$ (AUC) and $C(t_m)/\lambda_z^2 + C(t_m)*t_m/\lambda_z$ (AUMC), where $C(t_m)$ is the last concentration above the limit of quantification at time t_m and λ_z is the slope of the terminal elimination phase. Total clearance (CL) was calculated as dose/$AUC_{i.v.}$. The volume of distribution at steady state was calculated as V_{ss} = CL*AUMC/AUC. Results expressed in this study are presented as the mean ± S.E.M. Significant differences between values 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.

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