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Tissue Distribution and Clinical Monitoring of the Novel Macrolide Immunosuppressant SDZ-RAD and Its Metabolites in Monkey Lung Transplant Recipients: Interaction with Cyclosporine[1]

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ABSTRACT

We report the tissue distribution and clinical monitoring of the novel macrolide immunosuppressant SDZ-RAD [40-*O*-(2-hydroxyethyl)-rapamycin] and its metabolites in monkey lung transplant recipients as well as its interaction with cyclosporine as the Neoral formulation. After left unilateral lung transplantation, cynomolgus monkeys received by oral administration either 1) 1.5 mg/kg/day SDZ-RAD ($n = 4$); 2) 100 mg/kg/day cyclosporine ($n = 4$); 3) 0.3 mg/kg/day SDZ-RAD + 100 mg/kg/day cyclosporine ($n = 6$); 4) 1.5 mg/kg/day SDZ-RAD + 50 mg/kg/day cyclosporine ($n = 5$); or 5) SDZ-RAD and cyclosporine doses adjusted according to trough blood concentration measurements ($n = 6$). At the end of the observation period (usually 29 days after transplantation), and 24 h after the last doses, tissue samples were collected and analyzed with HPLC/mass spectrometry. Gall bladder, pancreas, the transplant lung, cerebellum, kidneys, and spleen had the highest SDZ-RAD concentrations. Coadministration of cyclosporine increased SDZ-RAD concentrations in most tissues as well as tissue-to-blood distribution coefficients. In contrast, SDZ-RAD had only a small effect on cyclosporine blood and tissue concentrations. Rejection in lung grafts in monkeys treated with either of the cyclosporine/SDZ-RAD combinations was significantly less than in the monotherapy groups ($P < .002$). Histological rejection scores were inversely correlated with SDZ-RAD concentrations in blood ($r = -0.68$; $P < .001$; $n = 24$), lymph nodes ($P = -0.58$; $P < .003$; $n = 24$), thymus ($r = -0.63$; $P < .001$; $n = 23$) and transplant lung tissue ($r = -0.58$; $P < .003$; $n = 24$). We conclude that, in addition to the synergistic pharmacodynamic interaction, a pharmacokinetic interaction resulting in higher SDZ-RAD tissue concentrations contributed to the significantly better immunosuppressive efficacy when both drugs were combined compared with monotherapy.

The novel macrolide SDZ-RAD [40-*O*-(2-hydroxyethyl)-rapamycin] is currently in phase II/III clinical trials as an immunosuppressant coadministered with microemulsion cyclosporine (Neoral; international nonproprietary name: ciclosporin) after organ transplantation. SDZ-RAD is a semisynthetic derivative of rapamycin (international nonproprietary name: sirolimus). Although not yet proven, it is assumed that SDZ-RAD has the same molecular actions as rapamycin. The rapamycin/FK-binding protein (FKBP) complex, and probably also the SDZ-RAD/FKBP complex, binds in T lymphocytes to mTOR, the mammalian target of rapamycin. The results are inhibition of the interleukin-2-stimulated phosphorylation activation of p70-kd S6 protein kinase and blockade of cell cycle progression at the G_1-S-interface (Schuler et al., 1997; Böhler et al., 1998). While the calcineurin inhibitor cyclosporine inhibits interleukin-2 synthesis, SDZ-RAD inhibits interleukin-2-mediated T-lymphocyte proliferation. It is generally thought that inhibition of the subsequent steps of T-lymphocyte proliferation is the main reason for the synergistic immunosuppressive interaction of cyclosporine and SDZ-RAD observed in in vitro and in vivo studies (Schuler et al., 1997; Schuurman et al., 1997, 1998). Major side effects of cyclosporine include nephrotoxicity, hepatotoxicity, neurotoxicity, and hypertension (Kahan, 1989). The side effect pattern of SDZ-RAD in patients can be expected to be similar to that of rapamycin (Murgia et al.,

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1996): thrombocytopenia, hyperlipidemia, and gastrointestinal disorders.

Access of cyclosporine to the various body compartments is governed by cytochrome P450 (CYP)3A, ATP-binding cassette protein transporters, and binding to immunophilins (Ryffel et al., 1991; Wacher et al., 1998). Like cyclosporine, SDZ-RAD is a substrate of both CYP3A and the ATP-binding cassette protein transporter p170-glycoprotein (Crowe and Lemaire, 1998; Crowe et al., 1999). Because many of the factors that determine cyclosporine pharmacokinetics also are involved in SDZ-RAD pharmacokinetics, we hypothesized that cyclosporine might have a significant effect on SDZ-RAD tissue distribution, thus influencing its immunosuppressive efficacy and its tolerability.

Due to the poor oral bioavailability of microemulsion cyclosporine in cynomolgus monkeys (Schuurman et al., 1996), significantly higher doses than in humans are required to maintain cyclosporine trough blood concentrations in the target range proposed for clinical lung transplantation. The cyclosporine (100 mg/kg) and SDZ-RAD (1.5 mg/kg) doses used in our study were based on those used in the same animal species by Schuurman et al. (1998). These doses resulted in trough blood concentrations in the therapeutic range for patients and were found to be effective in preventing rejection of life-supporting kidney transplants for >50 days. Because coadministration of 100 mg/kg cyclosporine and 1.5 mg/kg SDZ-RAD was tolerated poorly (Hausen et al., 2000), doses of 50 mg/kg cyclosporine and 1.5 mg/kg SDZ-RAD (for comparison with the SDZ-RAD monotherapy group) and of 100 mg/kg cyclosporine and 0.3 mg/kg (for comparison with the cyclosporine monotherapy group) were combined. These doses were based on the results of tolerability studies (Hausen et al., 2000). In addition to these fixed-dose drug regimens, we also included a study group in which, as in the clinical practice, doses were adjusted according to blood trough concentrations (20–40 μg/l for SDZ-RAD and 100–200 μg/l for cyclosporine). The cyclosporine target concentrations were based on those used in patients (Oellerich et al., 1995).

Experimental Procedures

Materials. SDZ-RAD, cyclosporine, and cyclosporin D were provided by Novartis Pharma AG (Basel, Switzerland). Acetonitrile (HPLC grade), sulfuric acid (American Chemical Society grade), methanol, and methylene chloride were obtained from Fisher Scientific (Fairlawn, NJ). Zinc sulfate, formic acid, and sodium formate (all American Chemical Society grade) were purchased from Sigma Chemical Co. (St. Louis, MO). Extraction columns (bonded phase C18; 1 ml) were from Varian Sample Preparation Products (Harbor City, CA). The internal standard used for quantification of SDZ-RAD, 28-,40-*O*-diacetyl rapamycin, was synthesized as described by Streit et al. (1996). Analytical columns (250 × 4 mm) filled with Hypersil (Shandon, Chadwick, UK) C8, 3-μm material were from Keystone Scientific (Bellefonte, PA). HPLC micro vials, 100-μl inserts, and Teflon screw caps were purchased from Hewlett Packard (Palo Alto, CA).

Samples were analyzed on an HPLC/electrospray-mass spectrometry (MS) system consisting of a series 1100 HPLC system (G1322A degasser, G1312A binary pump, G1313A autosampler, and G1316A column thermostate), a 59887A electrospray interface equipped with an Iris hexapole ion guide (Analytica of Brandford, Brandford, CT),

Lung Transplantation in Cynomolgus Monkeys. The animals received humane care in compliance with the Principles of Laboratory Animal Care (National Society for Medical Research) and the Guide for Care and Use of Laboratory Animals (National Academy of Sciences, published by the National Institutes of Health). The Institutional Animal Care and Use Committee of Stanford University granted approval.

Cynomolgus monkeys, *Macaca fascicularis*, imported by the Charles River Biomedical Research Foundation (Houston, TX) from Mauritius, were free of herpes B virus and Sendai virus. All animals were quarantined for a minimum of 2 months at Charles River and for 7 weeks at Stanford. While in quarantine, blood was drawn on each donor/recipient pair of animals for a mixed lymphocyte reaction assay after the protocol kindly provided by Dr. B. A. Cosimi (Harvard Medical School, Boston, MA). Donor and recipient monkeys were blood-group matched and mixed lymphocyte reaction mismatched to obtain a stimulation index of at least 2.5. After quarantine, left unilateral lung transplantation was carried out as described by Cooper (1989). For the first 24 postoperative hours, the monkeys were placed into intensive care cages supplied with oxygen and warm, humid air. They had free access to food and water 4 h after transplantation and were transferred to the regular animal room 1 day later. The animals were assigned to one of five treatment groups after transplantation: 1) 1.5 mg/kg/day SDZ-RAD (group 1.5RAD; $n = 4$); 2) 100 mg/kg/day cyclosporine (group 100Cs; $n = 4$); 3) 0.3 mg/kg/day SDZ-RAD + 100 mg/kg/day cyclosporine (group 0.3RAD + 100Cs; $n = 6$); 4) 1.5 mg/kg/day SDZ-RAD + 50 mg/kg/day cyclosporine (group 1.5RAD + 50Cs; $n = 5$); and 5) concentration-controlled dose adjustments based on SDZ-RAD and cyclosporine trough blood concentrations ($C_{24\,h}$, group CCRAD + Cs; $n = 6$). In the groups with 100 mg/kg/day cyclosporine, the monkeys initially received 150 mg/kg/day. On postoperative day 7, the dose was lowered to 100 mg/kg/day and remained unchanged for the remainder of the study period. In the concentration-controlled dosing group (CCRAD + Cs), doses were adjusted to maintain SDZ-RAD and cyclosporine trough blood concentrations in target concentration ranges of 20 to 40 μg/l and 100 to 200 μg/l, respectively. During the study period, the mean ± S.D. daily SDZ-RAD dose in this group was 0.6 ± 0.1 and 38 ± 1 mg/kg for cyclosporine. Immunosuppressive therapy was started immediately after surgery (day 0). Cyclosporine (Neoral) and SDZ-RAD (in methylcellulose vehicle) were given daily as a single oral dose. In addition, animals received a single dose of methylprednisolone (4 mg/kg i.v.) at the day of surgery, and the antibiotics cefazolin (25 mg/kg i.m.) and gentamycin (3 mg/kg s.c.) for the first 4 days after surgery. At either the end of the observation period (29 days after surgery) or when the animals' health status seriously deteriorated, and 24 h after the last dose, the animals were sacrificed and the following samples were collected for measurement of drug tissue distribution: blood, brain stem, cerebellum, cerebrum, colon, duodenum, fat, gall bladder, heart, ileum, jejunum, kidney, liver, transplant lung, native lung, lymph nodes, pancreas, spleen, stomach, testis, and thymus. The tissues were frozen in liquid nitrogen immediately after collection and stored at −80°C until HPLC/MS analysis. Samples were analyzed within 20 days.

Extraction of Tissue Samples. Tissue samples were thawed, weighed, and homogenized with 2 ml of KH_2PO_4 buffer [pH = 7.4 (1 M)] with a Teflon-glass manual homogenizer. One milliliter of homogenate was taken for analysis. Cyclosporin D and 28-,40-diacetyl sirolimus (in acetonitrile/sulfuric acid; pH = 3; 90:10 v/v) were added as internal standards, resulting in final concentrations of 100 μg/l of each. After addition of 2 ml of methanol/0.4 M $ZnSO_4$ (80:20 v/v) for protein precipitation, the samples were vortexed for 30 s and centrifuged at 1500*g* for 3 min. The organic supernatant was loaded on C18 extraction cartridges by drawing the samples through the columns with a −10 mm Hg vacuum. The extraction columns had previously been primed with 3 ml of water and 3 ml of acetonitrile. Immunosuppressants, metabolites, and internal standards were

were dried by drawing air for 5 min. Samples were eluted with 1.5 ml of methylene chloride. The samples were evaporated to dryness under a stream of nitrogen at 50°C. The residues were reconstituted in 120 μl of acetonitrile/0.1% formic acid (75:25 v/v) and were transferred into micro HPLC vials with conical 100-μl inserts and sealed with Teflon screw caps.

HPLC/Electrospray-MS Analysis. Extracts (100 μl) were injected onto a 250- × 4-mm analytical column filled with Hypersil C8-material of 3-μm particle size. SDZ-RAD, cyclosporine, their metabolites, and the internal standards were eluted from the analytical column with methanol and 0.1% formic acid supplemented with 1 μmol/l sodium formate. The following linear gradient was used: 0 min, 70% methanol; and 30 min, 90% methanol. The column was reequilibrated to starting conditions for 5 min before injection of the next sample. The flow rate was 0.4 ml/min and the column temperature was 65°C. The drying gas in the electrospray source was adjusted to a value of 42 (arbitrary units) and heated to 350°C. The pressure of the needle spray gas was 80 psi. The quadrupole was heated to 120°C. The mass spectrometer was run in the positive mode and the multiplier voltage was set to 1750 V and the X-ray voltage to −10 kV. For single ion detection, the mass spectrometer was focused on the $[M + Na]^+$ of SDZ-RAD [980 atomic mass units (amu)], hydroxy SDZ-RAD (996 amu), desmethyl SDZ-RAD (966 amu), the internal standard 28-,40-diacetyl sirolimus (1020 amu), cyclosporine (1224 amu), hydroxy cyclosporine (1240 amu), dihydroxy cyclosporine (1256 amu), desmethyl cyclosporine (1210 amu), and the internal standard cyclosporin D (1238 amu). The dwell time per ion was 100 ms.

Assay Validation and Quantification. The assay was validated for SDZ-RAD and cyclosporine in blood with the procedures described in detail by Segarra et al. (1998). The HPLC/MS assay had the following specifications for SDZ-RAD determined in blood: linear range 0.1 to 100 μg/l ($y = 0.96x + 0.05$; $r^2 = 0.99$), mean intra-assay variability 6.9% ($n = 10$), intraday accuracy +6.8% ($n = 10$), interassay variability 8.0% ($n = 6$; 3 days), and mean analytical recovery 83%. In-process stability, freeze-thaw stability, dilution integrity, and partial volume verification were established and have been reported by Segarra et al. (1998). The specifications for cyclosporine were as follows: linear range 1 to 1000 μg/l ($y = 0.93x + 15.1$; $r^2 = 0.98$), intra-assay variability 7.7% ($n = 10$), intraday precision −3.7% ($n = 10$), interassay variability 9.8% ($n = 5$; 3 days), and mean analytical recovery 86%. Abbreviated assay validations were carried out for each of the tissues, including analytical recovery, lower and upper limit of quantitation, linearity, interassay variability, and accuracy. Samples from tissues of untreated donor monkeys were collected. Tissue samples were homogenized and cyclosporine and SDZ-RAD were added and incubated at 37°C for 30 min to allow for distribution and protein binding. The following samples were prepared: blanks ($n = 3$/tissue), calibration controls (four concentrations; $n = 3$/concentration), precision controls (three concentrations; $n = 3$/concentration), samples for determination of the lower (SDZ-RAD, 0.5 μg/l and cyclosporine, 10 μg/l; $n = 5$) and upper limit of quantitation (SDZ-RAD, 100 μg/l and cyclosporine, 1000 μg/l; $n = 5$). The samples were extracted and analyzed as described above. Recoveries were calculated from the quality control samples ($n = 3$ for each concentration). The mass spectrometer responses of the extracted samples were compared with the response after injection of corresponding amounts of internal standard or with standard solutions of the immunosuppressants (in methanol/0.1% formic acid; 9:1 v/v) directly on the analytical column. The lowest concentration that met the following criteria was accepted as the lower limit of quantitation: 80% of the samples analyzed had to be within ±20% of the nominal value, and precision and accuracy variation had to be less than 20%. The upper limit of quantitation was determined similarly.

No interferences with the assay were detected when blank tissues were analyzed. Recoveries of SDZ-RAD and cyclosporine from all tissues were >60%. Validation results are summarized in Table 1. The validation results in all tissues were similar and did not significantly differ from those in blood. Therefore, tissue and blood concentrations of the immunosuppressants in the study samples were calculated with external calibration curves prepared in blood and were corrected with the internal standards. When tissue sample concentrations exceeded the upper limit of quantification, samples were diluted with acetonitrile/0.1% formic acid (75:25 v/v) and reanalyzed.

Cyclosporine and SDZ-RAD metabolites were identified by com-

TABLE 1

Validation of the HPLC/MS assay for cyclosporine and SDZ-RAD in monkey organ tissues

Tissue samples were collected from untreated monkeys. Tissues were homogenized and cyclosporine or SDZ-RAD was added and incubated at 37°C for 30 min to allow for distribution and protein binding. The samples were extracted as described in *Experimental Procedures*. A concentration was accepted as the lower or upper limit of quantitation when four of five samples (80%) were within ±20% of the nominal concentration and the coefficient of variation of all samples was ≤20%. The calibration curve consisted of four different concentrations ($n = 3$ for each concentration). Interassay coefficient of variation (CV) and accuracy (Acc) were measured at three different concentrations ($n = 3$ per concentration). Means ($n = 9$) are presented.

Tissue	Cyclosporine				SDZ-RAD			
	Linear Range	Regression Analysis	CV	Acc	Linear Range	Regression Analysis	CV	Acc
	μg/l		%		μg/l		%	
Brainstem	50–1000	$y = 1.02x - 7.5$, $r = 0.996$	3.0	−0.8	0.5–100	$y = 0.91x + 0.22$, $r = 0.999$	8.3	−5.5
Cerebellum	50–1000	$y = 1.04x - 9.5$, $r = 0.997$	5.7	−1.3	0.5–100	$y = 0.91x - 0.31$, $r = 0.999$	4.7	−2.4
Cerebrum	50–1000	$y = 0.98x - 4.6$, $r = 0.998$	5.9	−3.9	0.5–100	$y = 1.05x - 0.25$, $r = 0.995$	7.1	+1.4
Kidney	50–1000	$y = 1.01x - 4.1$, $r = 0.995$	9.8	+0.2	0.5–100	$y = 1.05x - 0.03$, $r = 0.997$	12.3	+6.0
Liver	50–1000	$y = 0.95x - 4.6$, $r = 0.992$	13.9	−10.3	0.5–100	$y = 1.04x - 0.37$, $r = 0.999$	9.8	+10.8
Stomach	50–1000	$y = 0.99x - 1.5$, $r = 0.993$	4.1	+1.1	0.5–100	$y = 1.05x - 1.38$, $r = 0.997$	7.9	−1.1
Colon	50–1000	$y = 1.01x + 3.9$, $r = 0.993$	6.9	+6.6	0.5–100	$y = 0.93x + 1.02$, $r = 0.997$	11.5	−2.8
Ileum	50–1000	$y = 1.01x - 5.7$, $r = 0.988$	6.5	−0.9	0.5–100	$y = 0.96x + 0.28$, $r = 0.997$	7.9	+2.8
Duodenum	50–1000	$y = 1.02x + 0.8$, $r = 0.990$	11.8	+10.2	0.5–100	$y = 1.01x + 0.42$, $r = 0.999$	11.5	−2.8
Jejunum	50–1000	$y = 0.91x + 0.8$, $r = 0.995$	6.0	−6.6	0.5–100	$y = 0.95x - 0.91$, $r = 0.997$	7.7	−2.7
Pancreas	50–1000	$y = 0.96x + 5.1$, $r = 0.998$	9.2	−6.6	0.5–100	$y = 1.06x - 1.67$, $r = 0.996$	15.4	−6.1
Heart	50–1000	$y = 1.07x - 5.9$, $r = 0.996$	6.8	+5.5	0.5–100	$y = 1.00x - 0.69$, $r = 0.999$	9.0	−5.6
Lung (right)	50–1000	$y = 1.02x - 2.6$, $r = 0.999$	7.4	+0.4	0.5–100	$y = 1.03x - 0.02$, $r = 0.997$	7.6	−2.7
Lung (left)	50–1000	$y = 1.06x + 2.2$, $r = 0.998$	6.0	+8.6	0.5–100	$y = 0.90x + 1.75$, $r = 0.997$	4.5	−3.7
Spleen	50–1000	$y = 1.07x - 2.1$, $r = 0.997$	8.0	+7.2	0.5–100	$y = 1.03x - 0.46$, $r = 0.999$	4.3	−0.8
Testes	50–1000	$y = 1.04x - 2.7$, $r = 0.995$	4.4	+4.7	0.5–100	$y = 0.99x - 1.25$, $r = 0.999$	3.6	−2.3
Thymus	50–1000	$y = 1.06x - 5.3$, $r = 0.996$	6.3	+3.5	0.5–100	$y = 0.96x - 0.5$, $r = 0.998$	8.0	+3.0
Fat	50–1000	$y = 1.02x - 1.4$, $r = 0.996$	3.6	+3.0	0.5–100	$y = 0.95x - 0.43$, $r = 0.996$	5.1	−3.4
Lymph node	50–1000	$y = 1.04x - 1.5$, $r = 0.995$	5.5	+2.7	0.5–100	$y = 0.98x \pm 0$, $r = 0.999$	3.8	−4.3
Gall bladder	50–1000	$y = 1.08x - 7.8$, $r = 0.999$	10.9	+2.1	0.5–100	$y = 1.11x - 0.47$, $r = 0.999$	9.3	−8.1

parison of the mass spectra and HPLC retention times with those of authentic standard material. Cyclosporine metabolites were isolated and their structures identified as previously described (Christians et al., 1991). SDZ-RAD was incubated with human liver microsomes and an NADPH-generating system. The metabolites were isolated by HPLC and their structures identified by MS and analysis of the fragment pattern after induction of nozzle-skimmer fragmentation as described by Vidal et al. (1998). Validation results for the quantification of metabolites were similar to those of the parent compounds.

Clinical Monitoring of Transplant Animals. Laboratory screening was performed three times a week and included differential blood counts, blood chemistry (serum lipid patterns, serum protein, liver and kidney function parameters), and serum electrolytes. Lung transplant function and rejection was monitored by 1) chest radiographs (two times per week), 2) lung function tests with a Bicore CP-100 pulmonary monitor (Bicore Monitoring Systems, Irvine, CA), 3) bronchoscopy (day 4 and day 14 after surgery), and 4) open lung biopsies 2 and 4 weeks after transplantation. The histological grade of rejection was classified according to the International Society for Heart and Lung Transplantation (A0, no rejection; A1, minimal rejection; A2, mild rejection; A3, moderate rejection; and A4, severe rejection; Yousem et al., 1996).

Data Analysis. Data were processed with ChemStation software revision A04.02 for the HPLC system and C.03.00 for the electrospray interface and MS (all from Hewlett-Packard). Concentrations of the immunosuppressants were calculated from an external standard curve and corrected on the basis of the internal standards. SPSS software, version 9.0, was used for statistical analysis (SPSS Inc., Chicago, IL). Because data were not normally distributed, tissue concentrations in the combination therapy and respective control groups were compared with the nonparametric Mann-Whitney *U* test and results of distribution statistics are reported as median and range (minimum–maximum). Clinical chemical and biochemical data among groups were compared by multivariate ANOVA. Correlation (Pearson correlation coefficients, two-tailed test of significance) and stepwise regression analysis (probability-of-*F*-to-enter, .05) were based on the data of all study groups ($n = 25$).

Results

Tissue Distribution of SDZ-RAD, Cyclosporine, and Their Metabolites

Tissue Distribution of SDZ-RAD (Group 1.5RAD). After single daily oral SDZ-RAD doses (1.5 mg/kg) for 4 weeks and 24 h after the last dose, the blood concentrations of SDZ-RAD at the time of sacrifice ranged from 3.7 to 44 μg/l (median = 9.2 μg/l) and those of its metabolites from 0 to 7.1 μg/l (median = 2.3 μg/l; $n = 4$; Fig. 1). SDZ-RAD and its metabolites extensively distributed into tissues (Fig. 1; Table 2). Median concentrations of SDZ-RAD and median total concentrations of its metabolites in most tissues exceeded those in blood. However, concentrations varied widely and, thus, the only organs that reached significantly higher tissue concentrations than in blood were pancreas and gallbladder ($P < .05$). Although there was a tendency to a higher median SDZ-RAD concentration in the transplant versus the native lung (Fig. 1), this difference was not statistically significant. It must be taken into account that due to the relatively small number of animals in the study groups, the statistical power for this analysis as well as all comparisons of the tissue concentrations reported below was less than 35%.

Tissue Distribution of Cyclosporine (Group 100Cs). Cyclosporine was detected in all organ tissues examined in

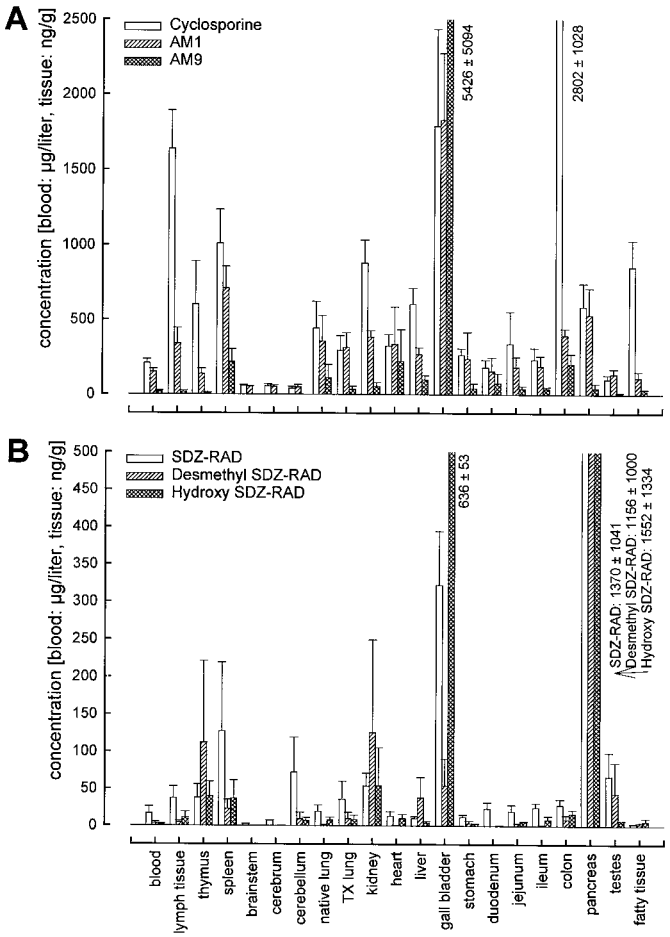

Fig. 1. Distribution of cyclosporine (A), SDZ-RAD (B), and their main metabolites in tissues of lung transplant monkeys. Data are presented as mean ± S.E. ($n = 4$) and are given in nanograms per gram of wet tissue weight except for blood, which is given in micrograms per liter. The animals in A received 100 mg/kg/day (150 mg/kg the first week) cyclosporine (Neoral) and the animals in B received 1.5 mg/kg/day SDZ-RAD as a single daily oral dose for 4 weeks. TX, transplant.

the median blood concentration of cyclosporine was 221 μg/l (range 131–255 μg/l; $n = 4$). Cyclosporine concentrations in the native and transplant lung were similar (Fig. 1). The cyclosporine metabolites AM1 and AM9 were present in all tissues, except AM9 was not detectable in brain tissues. Median cyclosporine metabolite concentrations, except in gall bladder and cerebrum, were lower than cyclosporine concentrations. The concentrations of cyclosporine and its metabolites exceeded those in blood in most tissues (Table 2). Again, mainly due to the high variability, only cyclosporine concentrations in fat tissue, kidney, spleen, lymph nodes, gall bladder, and colon were significantly higher than in blood ($P < .05$).

Effect of Cyclosporine on Tissue Distribution of SDZ-RAD (Group 1.5RAD + 50Cs). Compared with the administration of each drug alone, combination of SDZ-RAD and cyclosporine affected the tissue concentrations of both drugs. As shown in Fig. 1B, SDZ-RAD concentrations had the tendency to increase in most tissues when coadministered with 50 mg/kg/day cyclosporine (group 1.5RAD + 50Cs) compared with tissues from monkeys in the corresponding SDZ-RAD monotherapy group (group 1.5RAD). In contrast to most

TABLE 2

Comparison of tissue-to-blood distribution coefficients of SDZ-RAD and its metabolites with (50 mg/kg, group 1.5RAD + 50Cs) and without cyclosporine (group 1.5RAD) and of cyclosporine and its metabolites with (0.3 mg/kg/day, group 0.3RAD + l00Cs) and without SDZ-RAD (group 100Cs)

The tissue-to-blood distribution coefficients were calculated by dividing the median concentration in tissue (nanograms per gram) by the median concentration in blood micrograms per liter.

	SDZ-RAD		Metabolites		Cyclosporine		Metabolites	
	−Cs	+Cs	−Cs	+Cs	−RAD	+RAD	−RAD	+RAD
Brainstem	0.1	0.5	0.0	0.2	0.3	0.5	0.3	0.2
Cerebellum	4.3	1.1	3.3	0.2	0.2	0.4	0.3	0.3
Cerebrum	0.4	1.1	0.0	0.6	0.3	0.8	0.3	0.1
Colon	1.7	5.8	5.1	2.9	13.5	3.5	6.1	0.8
Duodenum	1.4	1.4	0.1	1.4	0.9	2.2	2.5	0.8
Fat	0.2	1.1	1.7	0.5	4.1	1.5	1.0	0.3
Gall bladder	19.4	27.8	140.6	23.3	8.6	9.9	67.6	35.1
Heart	0.8	1.1	2.1	1.0	1.6	1.4	3.9	0.8
Ileum	1.5	6.5	1.8	4.9	1.1	3.0	2.2	1.3
Jejunum	1.2	3.2	1.8	3.8	1.6	3.5	2.1	2.1
Kidney	3.2	9.7	36.6	12.2	4.3	3.9	2.7	3.0
Liver	0.6	2.2	8.6	2.8	2.9	2.1	3.1	3.4
Lung (native)	1.2	2.1	1.9	1.6	1.4	1.0	2.1	1.0
Lung (tx)	12.6	1.7	21.4	3.5	2.1	2.0	3.3	2.2
Lymph node	2.2	2.4	3.2	0.6	7.9	15.6	2.0	0.8
Pancreas	82.6	8.8	551.4	3.7	2.8	1.9	5.5	1.5
Spleen	7.7	10.6	12.5	9.1	4.9	2.6	6.8	4.2
Stomach	0.7	1.8	1.3	0.7	1.3	1.4	2.1	0.6
Testes	4.0	1.7	10.0	0.5	0.5	0.4	0.8	0.4
Thymus	2.3	1.7	30.9	1.2	2.9	2.3	2.4	0.7

Cs; cyclosporine, RAD; SDZ RAD, tx, transplant.

pancreas and cerebellum were lower in the presence of cyclosporine than in the SDZ-RAD monotherapy group (Fig. 2). This was because, compared with the other animals in this group, one monkey in the SDZ-RAD monotherapy group (1.5RAD) had extremely high concentrations in these organs (Fig. 1). Compared with the monotherapy group, with concomitant cyclosporine the changes of SDZ-RAD concentrations in blood, ileum, duodenum, native lung, kidney, and brainstem (Fig. 1B) reached statistical significance (all $P <$ .04). As indicated by the tissue-to-blood partition coefficients [C_{tissue} (micrograms per gram)/C_{blood} (micrograms per liter)], the interaction with cyclosporine led to an increase of SDZ-RAD concentrations in most tissues, the extent of which was not predicted by blood concentrations (Table 2). In most tissues (Table 2; Fig. 2), cyclosporine increased the SDZ-RAD/metabolite ratio. Statistically significant correlations of SDZ-RAD trough blood concentrations with tissue concentrations were only observed in 10 of 20 tissues examined, and significant correlations for blood and tissue concentrations of SDZ-RAD metabolites were only observed in 2 of 20 tissues (Table 3).

Effect of SDZ-RAD on Tissue Distribution of Cyclosporine (Group 0.3RAD + 100Cs). SDZ-RAD had a smaller impact on cyclosporine tissue distribution than the inverse (Fig. 1, A and B, respectively). The Mann-Whitney *U* test did not show any statistically significant changes, including concentrations in blood and the transplant lung. Comparing the changes in all tissues, the effect of SDZ-RAD on median cyclosporine tissue/blood ratios lacked a consistent tendency (Table 2). In general, trough blood concentrations of cyclosporine and its metabolites showed a much better correlation with their tissue concentrations than SDZ-RAD and its metabolites (Table 3).

Lung Transplant Rejection, Toxicity, and Clinical Monitoring (Table 4)

Group 1.5RAD (1.5 mg/kg/day SDZ-RAD). At the end of [illegible] rejection (A4) of the transplant lung. The median weight loss at the end of the study was 22% (Table 4). There were no signs of anemia or thrombocytopenia. Serum cholesterol concentrations increased during the observation period (Table 4) but were not significantly different from the cyclosporine control group (group 100Cs).

Group 100Cs (100 mg/kg/day Cyclosporine). At the end of the study, the transplant lungs in two animals showed moderate (A3) and in the other two animals severe rejection (A4). The median weight loss during the study was 15%. Serum cholesterol concentrations increased during the observation period but the change was not statistically significant. No other changes in clinical chemical and biochemical parameters were detected.

Group 0.3RAD + 100Cs (0.3 mg/kg/day SDZ-RAD + 100 mg/kg/day Cyclosporine). The combination of SDZ-RAD and cyclosporine was more effective in preventing rejection of the lung allografts than monotherapy of either drug. At the end of the study, the biopsies of five animals showed a rejection score of A2 (mild acute rejection) and the biopsy of one animal had a rejection score of A3 (moderate acute rejection). However, in contrast to the monotherapy groups 1.5RAD and 100Cs, the animals developed significant anemia. Three animals required erythropoetin treatment and two animals required blood transfusions. Platelet counts were as low as 37,000/μl. These changes are not reflected in Table 4 because the table only includes the last values before sacrifice. From postoperative day 15 onward, serum cholesterol concentrations were higher in this group than in the control groups. However, the differences did not reach statistical significance. The median weight loss in this group during the observation period was 22% (Table 4). Three of six animals had to be sacrificed early due to anemia and renal failure (n = 2; days 20 and 23) or seizures (n = 1; day 22).

Group 1.5RAD + 50Cs (1.5 mg/kg/day SDZ-RAD + 50 [illegible]

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