

# SDZ RAD, A NEW RAPAMYCIN DERIVATIVE: Synergism with Cyclosporine

[Experimental Transplantation]

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## Abstract

**Background.** SDZ RAD is a new rapamycin analog with potent immunosuppressive activity. Compounds of the rapamycin class differ in their mode of action from cyclosporine, thus providing a rationale for potential synergism of these two potent immunosuppressants.

**Methods.** The two-way mouse mixed lymphocyte reaction (BALB/c-CBA strain combination) was applied. Orthotopic kidney and heterotopic heart allografting was performed in the stringent DA-to-Lewis rat strain combination, with administration of compounds orally as microemulsion concentrate (i.e., Neoral in the case of cyclosporine).

**Results.** Isobologram analysis of checkerboard titrations of SDZ RAD and cyclosporine in two-way mouse mixed lymphocyte reactions indicates a synergistic interaction in vitro. In vivo, the minimal effective dose of microemulsion cyclosporine giving long-term graft survival was 5.0 mg/kg/day; for SDZ RAD, the minimal effective dose was 5.0 mg/kg/day in kidney transplantation and >5.0 mg/kg/day in heart transplantation. Long-term allograft survival was noted for combinations of microemulsion cyclosporine administered at 1.0 or 2.0 mg/kg/day and SDZ RAD given at between 0.5 and 2.0 mg/kg/day. The index of synergy in different combinations ranged between 0.3 and 0.7.

**Conclusions.** SDZ RAD and cyclosporine show synergism in immunosuppression, both in vitro and in vivo. They form a promising synergistic drug combination in allotransplantation.

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There is increasing interest in the immunosuppressant rapamycin, a macrolide antibiotic produced by *Streptomyces hygroscopicus* (1, 2). A main complication encountered in the development of rapamycin is a proper formulation to cope with the wide interindividual variation in pharmacokinetic properties observed upon oral administration either in animals or humans (3, 4). In a preclinical program designed to overcome the formulation problems of rapamycin while maintaining the pharmacological activities, we identified a new rapamycin analog, SDZ RAD (40-O-(2-hydroxyethyl)-rapamycin, C<sub>53</sub>H<sub>83</sub>NO<sub>14</sub>, molecular weight 958). In our report on the pharmacological in vitro and in vivo characteristics of the new compound (this issue, 5), we demonstrate that the oral activity of SDZ RAD is at least equivalent to that of rapamycin.

The mechanism of action of rapamycins differs from that of cyclosporine, the immunosuppressant that generally forms the basic component in drug combinations used to prevent or inhibit allograft rejection. While cyclosporine acts early after T-cell activation, blocking transcriptional activation of early T cell-specific genes, rapamycin acts later in the cell cycle by blocking growth factor-driven cell proliferation. This difference provides a rationale for synergism of rapamycin and cyclosporine, which indeed has been demonstrated in vitro for lymphocyte proliferation (6, 7) and in vivo in rodent models of transplantation and autoimmune disease (8-11). Because cyclosporine and rapamycin differ in their respective side effect profiles (12), this synergistic interaction may potentially widen the therapeutic window of each individual compound in combination treatment. This has been documented in organ allografting in dogs (13, 14) and cynomolgus monkeys (12). Therefore, we addressed the potential synergistic action between SDZ RAD and cyclosporine in rodents, both in vitro in murine mixed lymphocyte reaction and in vivo in transplantation models in the rat. The evaluation of synergy was based on a method proposed by Berenbaum (15), which was extended with an interaction term to correct for the different mechanism of action of the two drugs (16). The index of synergy was calculated as: Equation in which the doses of compounds *A* and *B* represent those used in a particular combination, and *A<sub>E</sub>* and *B<sub>E</sub>* are the equieffective doses of *A* or *B* giving the same effect at single treatment. If the result is less than 1, synergy can be concluded; *A* and *B* are additive if the index is 1 and antagonistic if the value is >1.

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$$\frac{\text{Dose of A}}{A_E} + \frac{\text{Dose of B}}{B_E} + \frac{(\text{Dose A}) \times (\text{Dose B})}{A_E \times B_E} \quad \text{Equation 1A}$$

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The two-way mouse mixed lymphocyte reaction was used for the in vitro evaluation of a potential synergistic interaction of SDZ RAD and cyclosporine. Spleen cells of BALB/c and CBA mice ( $1 \times 10^5$  from each strain) were cultured in duplicate in flat-bottom 96-well microtiter plates in the absence or presence of the serially diluted compound or combinations of the two compounds. Serum-free tissue culture medium supplemented with serum replacement factors was used (GC medium, Camon GmbH, Wiesbaden, Germany). After 4 days, [ $^3\text{H}$ ]-thymidine was added. Sixteen hours later, the cells were harvested and [ $^3\text{H}$ ]-thymidine incorporation was measured by liquid scintillation counting. The maximum [ $^3\text{H}$ ]-thymidine incorporation in the absence of any inhibitors was around  $240 \times 10^3$  cpm, and the background [ $^3\text{H}$ ]-thymidine incorporation in nonstimulated cells was around  $5 \times 10^3$  cpm. Drug concentrations at which the maximum proliferative response (i.e., [ $^3\text{H}$ ]-thymidine incorporation) was inhibited by 70% ( $\text{IC}_{70}^*$ ) were calculated using a four-parameter logistic function. This gave an  $\text{IC}_{70}$  of 21 nM for cyclosporine and 0.3 nM for SDZ RAD. To evaluate the effect of drug combinations, lymphocyte proliferation was determined in the presence of 0.05, 0.1, 0.2, 0.4, and 0.8 nM SDZ RAD, together with 2.5, 5.0, 10, 15, 20, or 25 nM cyclosporine. For each drug combination, the respective  $\text{IC}_{70}$  of each individual compound was calculated.  $\text{IC}_{70}$  values were then converted into relative units with regard to the  $\text{IC}_{70}$  value of the respective single drug. These relative  $\text{IC}_{70}$  units are graphically presented in an isobologram (Fig. 1). The concave nature of this isobologram indicates a synergistic interaction between the two compounds.

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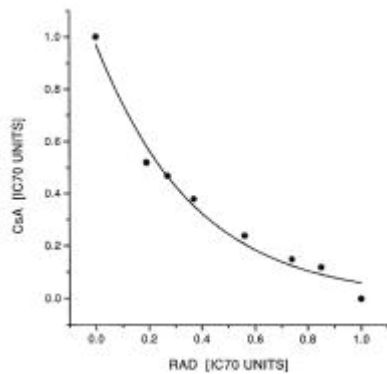


Figure 1. Isobologram of relative  $IC_{70}$  of cyclosporine and SDZ RAD in combination. The  $IC_{70}$  was 21 nM for cyclosporine alone and 0.3 nM for SDZ RAD alone.

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In vivo studies were performed in accordance with the Swiss Animal Welfare Act dated March 9, 1978, and the accompanying Animal Welfare Regulation of May 28, 1981. We performed orthotopic kidney or heterotopic heart allotransplantation in male Lewis rats (RT1<sup>b</sup> haplotype), using donor organs from male DA rats (RT1<sup>a</sup> haplotype). Kidney transplantation was followed by contralateral nephrectomy 7 days later. At nephrectomy, the graft was macroscopically inspected. If rejection was macroscopically evident, the experiment was terminated. Surviving animals were monitored daily for clinical signs of renal dysfunction. The heart allograft was transplanted into the abdomen, with anastomoses between the donor aorta and the recipient infrarenal abdominal aorta and between the donor right pulmonary artery and the recipient inferior vena cava. This was followed by daily palpation of the abdomen for a beating graft; in case of cessation of heart beat, the experiment was terminated. In both kidney and heart allograft experiments, the termination point in long-term survivors was 100 days. In all cases, the graft was removed at autopsy, fixed in buffered formalin, and embedded in paraffin. Four-micrometer-thick sections stained with hematoxylin and eosin were read for signs of rejection. Rejection was scored either as absent or as marginal, slight, moderate, or severe cellular rejection based on the extent of mononuclear cell infiltration and damage to the parenchyma (tubules in the kidney, myocytes in the heart). Lewis rats left untreated after transplantation rejected a kidney allograft within 7 days, with histology of severe cellular rejection; the heterotopic heart allograft in untreated recipients stopped beating between days 7 and 10 after transplantation, with a similar histology of severe cellular rejection (data not shown).

Cyclosporine, SDZ RAD, or both were given daily orally as microemulsion preconcentrates, which are optimized for the respective compound (i.e., for cyclosporine, this microemulsion

preconcentrate is Neoral, Novartis Pharma Ltd., Basel, Switzerland). Allograft survival data and histology are presented in Table 1. The minimum effective dose of microemulsion cyclosporine that gave long-term allograft survival was 5.0 mg/kg body weight, with slight rejection in graft histology. For SDZ RAD, a dose of 5.0 mg/kg had to be given to yield long-term survival of a kidney allograft; two recipients with  $\geq 100$  days of allograft survival showed marginal signs of rejection, and one case that rejected at day 42 after transplantation showed moderate cellular rejection in histology. In heart transplantation, a dose of 5.0 mg/kg SDZ RAD was insufficient to achieve long-term graft survival; in two recipients, the allograft stopped beating at 22 and 33 days after transplantation, and moderate cellular rejection was shown in histology. A higher SDZ RAD dose was not be given because higher SDZ RAD doses are associated with severe loss of body weight.

Microemulsion Cyclosporine, mg/kg	SDZ RAD, mg/kg	n	Survival (days)	Rejection (histology)
5.0	0.5	4	21, 21, 21, 21	Marginal
5.0	1.0	4	181, 181, 181, 181	Marginal
5.0	2.0	4	181, 181, 181, 181	Marginal
2.0	0.5	4	181, 181, 181, 181	Marginal
2.0	1.0	4	181, 181, 181, 181	Marginal
2.0	2.0	4	181, 181, 181, 181	Marginal
1.0	0.5	4	181, 181, 181, 181	Marginal
1.0	1.0	4	181, 181, 181, 181	Marginal
1.0	2.0	4	181, 181, 181, 181	Marginal
0.5	0.5	4	181, 181, 181, 181	Marginal
0.5	1.0	4	181, 181, 181, 181	Marginal
0.5	2.0	4	181, 181, 181, 181	Marginal

Table 1. Effect of microemulsion cyclosporine and SDZ RAD in rat transplantation

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In combination treatment, a dose of 1.0 or 2.0 mg/kg microemulsion cyclosporine was combined with 0.5, 1.0, or 2.0 mg/kg SDZ RAD. For the 1.0 mg/kg microemulsion cyclosporine dose, combinations with 0.5 mg/kg SDZ RAD proved sufficient to achieve long-term survival of a kidney allograft, whereas a minimum dose of 1.0 mg/kg was required to achieve long-term heart allograft survival. The higher dose required for heart allograft survival fits with the observation in single treatment, in which a dose of 5.0 mg/kg SDZ RAD yielded long-term survival of a kidney allograft but was less effective in heart allograft survival. The efficacy of combination treatment was also demonstrated by the histology of long-surviving allografts. Marginal to slight signs of rejection were observed in combinations of 1.0 mg/kg microemulsion cyclosporine with 0.5, 1.0, and 2.0 mg/kg SDZ RAD in kidney transplantation, and of 1.0 mg/kg microemulsion cyclosporine with 1.0 and 2.0 mg/kg SDZ RAD in heart transplantation. In all combinations of SDZ RAD with 2.0 mg/kg microemulsion cyclosporine, long-surviving kidney and heart

allografts did not reveal any sign of rejection in histology. Based on a minimal effective dose giving long-term survival in single compound treatment (5.0 mg/kg for microemulsion cyclosporine and  $\geq 5$  mg/kg for SDZ RAD), indexes of synergy in combination treatment were calculated. For kidney transplantation, the values were 0.3 (1.0 mg/kg microemulsion cyclosporine) and 0.5 (2.0 mg/kg microemulsion cyclosporine); for heart transplantation, they were  $< 0.5$  (1.0 mg/kg microemulsion cyclosporine) and  $< 0.7$  (2.0 mg/kg microemulsion cyclosporine).

From these data, we conclude that SDZ RAD and cyclosporine act synergistically in prevention of alloreactivity, either in vitro in the mouse mixed lymphocyte reaction or in vivo in rat transplantation models. As cyclosporine and macrolides differ in their respective side effect profile, this synergism might provide a larger therapeutic window for determining the doses of the compounds. Lowered drug doses in combination treatment are illustrated by the present data on heart allografting, in which the maximum dose of 5.0 mg/kg SDZ RAD tolerated by the animal was insufficient to yield long-term survival, whereas a fivefold lower dose in combination with 1.0 mg/kg microemulsion cyclosporine yielded long-term survival. The present synergy opens promising perspectives for clinical transplantation using drug combinations of SDZ RAD and microemulsion cyclosporine to suppress alloreactivity.

**Footnotes** [\[Context Link\]](#) 

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