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Letter to the Editor

Identification of a new pharmacologic action for an old compound

We report for the first time that rapamycin (RPM), a novel macrolide fermentation product originally developed as an antifungal agent, prolongs the survival of organ grafts exchanged between highly histoincompatible strains of rodents without apparent drug-related toxicity.

As a result of largely empirical work over the last three decades, transplantation has slowly gained respectability and is now the preferred treatment for several serious diseases. Despite recent advances, however, transplantation is an imperfect therapy that is far from realizing its full potential. Today transplantation of cadaveric grafts is still an expensive, labour-intensive treatment for a relatively small number of patients who still must endure an unacceptably high incidence of morbidity and mortality related to rejection, infection, malignancy, and drug toxicity that prevents complete rehabilitation for these patients. The best that transplantation can offer is palliation – not a cure. All of the problems that continue to haunt the field of transplantation stem from a single root cause: our inability to control the recipient's immune response to the graft effectively, safely, selectively and inexpensively.

Despite both the central importance of immunosuppressive drug development to continued progress in transplantation and the rapid accumulation of our knowledge of the immune system, it is noteworthy that clinical transplantation must rely only only four drugs (two of which were introduced a generation ago) to control rejection. To increase the chances of identifiying new lead compounds that could be developed into clinically important immunosuppressive therapeutics, our laboratory has begun a systematic search for new compounds. FK506 (FK) is a promising new immunosuppressive compound that we [1] and others [2] have begun to investigate. Although its immunosuppressive actions are similar to cyclosporine (CsA), FK is a macrolide and is, therefore, structurally distinct from CsA. It is still too early to predict what role this new, potent and very effective agent will play in clinical transplantation since it is still in the formative stages of development.

RPM (AY-22,989) was discovered in the mid-1970s at Wyeth-Ayerst Research Laboratories, found to have antifungal activity and, like FK, is a macrolide fermentation product [3]. As part of routine animal toxicological studies, high doses of RPM were found to alter lymphoid tissue histology, and subsequent studies in rats showed that RPM was also an effective treatment of experimentally-induced autoimmune disease [4]. This immunosuppressive activity of RPM discouraged its development as an antifungal agent, and although there was some interest in its antitumour activity, the molecule remained a potential solution in search of an appropriate clinical problem.

Several lines of evidence made a compelling case for the investigation of RPM's utility as an immunosuppressant for T-cell mediated allograft rejection: (1) RPM is stucturally similar to FK and FK is a proven inhibitor of T-cell activation and graft rejection, (2) RPM suppresses T-cell mediated autoimmune diseases, (3) RPM appears to have a wide margin of safety since the acute LD$_{50}$ dose of RPM in rats is

RPM was capable of prolonging the survival of transplanted tissue, two murine models of organ transplantation were used. In the first model, we determined the survival times of donor BALB/c (H-2^d) mouse neonatal hearts transplanted into the ear pinnae of recipient C3H/Km (H-2^k) mice. The technique was a modification of the method we have used to quantitate immunosuppression by cyclosporine [6, 7]. The recipient control group consisted of untreated and saline treated animals. Recipients in the RPM treatment group were treated daily from post operative day 1 through 13 by gavage (PO) with powdered RPM (provided by Wyeth-Ayerst Research, Princeton, NJ) suspended in 2% carboxymethylcellulose in water. Each heart graft was examined visually every other day from day 6 until day 14 and then Monday, Wednesday and Friday until the day of rejection (final day of graft survival) which was defined as the first day on which no graft contractile activity was seen.

In the second model, Brown Norway (RT1^b) rat hearts were transplanted by primary vascular anastomoses into the abdomens of Lewis (RT1^l) rat recipients as previously described [8]. Recipients in the control group were treated with normal saline intraperitoneally (IP) daily from post operative day 1 until the grafts rejected. Recipients in the treatment group were treated daily by gavage with RPM formulated as described above from post operative day 1 until day 14. Each heart graft was palpated daily until the day of rejection which was defined as the day on which graft contractile activity had ceased as confirmed visually at laparotomy.

For comparative purposes, mouse and rat recipients in certain groups were treated orally with cyclosporine diluted in olive oil using a treatment schedule identical to that used for RPM. In both murine models the donors and recipients are incompatible across the major histocompatibility complex. The Mann-Whitney U-test with a correction for small sample sizes was used to determine the statistical significance of the differences in graft survival times between groups.

Table 1 shows the results of the investigation of the initial use of RPM as an immunosuppressant for graft rejection. An oral dose of 6 mg kg^{-1} of RPM administered for only the first 13 post-transplant days enabled BALB/c hearts transplanted into C3H recipients to survive a median of 15 days. Compared with the graft survival times in the saline/no treatment group, the prolongation of graft survival in mice treated by RPM was highly significant ($p < 0.0001$). Even though CsA was significantly ($p < 0.013$) less effective than the dose of RPM that was half the CsA prolonged graft survival significantly compared with the control group, CsA dose.

Rat recipients were also susceptible to the antirejection activity of RPM, since the heart grafts in the RPM-treated group survived significantly ($p < 0.001$) longer than grafts in those animals treated with saline. Unlike our results in the mouse, we did not find that RPM was a more potent immunosuppressant than CsA for rat allograft rejection. Micronization of the RPM, formulation in an improved

Table 1: Prolongation of heart graft survival by orally administered (RPM) and cyclosporine (CsA).

Species	Treatment	Dose (mg kg^{-1})	Individual survival times (days)	Group survival times (days) Mean ± 95% confidence limits	Median	*p* values	
Mouse[a]	Saline	—	10(×74),12(×29),14(×2)	10.6 ± 0.2	10		
Mouse	CsA	12	8,10,12(×5),14	12 ± 1.5	12	=0.026	<0.0001
Mouse	RPM	6	10,12,12,14,14,16(×4),19	14 ± 1.9	15	=0.013	
Rat[b]	Saline	—	6,7(×6)	7 ± 0.35	7		
Rat	CsA	2	9,24,24,30	22 ± 14	24	=0.003	=0.001
Rat	RPM	3	15,22,22,29,31	24 ± 7.9	22	=1.0	

[a]*BALB/c to C3H;* [b]*Brown Norway to Lewis;* [c]*Mann-Whitney U-test.*

immunosuppressive activity of RPM, few conclusions can be made with certainty. The data do show that RPM prolongs the survival of highly histoincompatible heart grafts in mouse and rat transplant recipients. As the acute LD_{50}'s for orally administered RPM are so high for the mouse and rat [9, 5], it was not surprising that the doses of RPM that we used to prolong graft survival caused no noticeable signs of toxicity.

RPM is now the second macrolide to show sufficient immunosuppressive activity to prolong the survival of transplanted tissue. The true mechanisms of immunosuppressive action of RPM are not known, but it is tempting to speculate that RPM and FK control the rejection response in similar ways if only because they share certain similarities in their molecular structure. Ultimately, the clarification of the contrasting mechanisms of immunosuppressive action of these macrolides and CsA may allow us to understand why these fungal fermentation products are so specifically suited to the control of the mammalian immune response.

In a more practical vein, further investigation of RPM is needed to define its potency, efficacy, selectivity and safety so that its potential for clinical use can be assessed and compared to the drug profiles of other known immunosuppressive agents. Comprehensive studies of this nature are ongoing in our laboratory; we now have evidence that transient treatment with RPM at doses higher than the doses used here or given by different routes induces indefinite unresponsiveness in recipients.

The route from the discovery of a new lead compound to the final clinical use is often long, treacherous and tortuous. Although the trail leading to the use of RPM as an antifungal agent has grown cold, the fresh scent of this compound's antirejection activity deserves attention. The apparent safety of the drug at doses that are immunosuppressive is reason for cautious optimism that RPM may contribute in some way to the solution of the serious problems that continue to plague clinical transplantation.

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Note added in proof: We have learned from Wyeth-Ayerst that when R.Y. Calue's group in Cambridge, England, was provided with RPM, they too found that the compound prolonged the survival at allografts in animals.

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