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A comparison of the effects of rapamycin and cyclosporine on kidney and heart morphology in a rabbit heterotopic heart transplant model

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Summary. Rapamycin (RAPA) or cyclosporine (CsA) was administered intravenously, daily for 60 days, to rabbits with heterotopic heart transplants. Groups of 5 rabbits were randomly assigned to receive RAPA at 0.05, 0.1, 0.5 or 1.0 mg/kg/day or CsA at either 5.0, 10.0 or 15 mg/kg/day. Drug vehicle and saline controls were also included. Animals were examined daily and the cervical allografts assessed by palpation for viability/rejection. In those animals in which the heart stopped beating, the heart was removed and processed for light microscopic evaluation. The duration of the study was for 60 days at which time the animals were sacrificed and the transplanted heart and native kidneys removed and processed for light microscopic assessment of rejection and drug toxicity respectively. Biochemical and functional parameters in these animals were previously reported (Transplantation 5: 340-345, 1993). Animals that rejected their grafts were maintained on the drug until the endpoint of the study to assess toxicity in the native kidneys. The rejected hearts from these animals were also harvested for microscopic evaluation. The results of the study revealed that heart rejection in drug treated animals was significantly lower than in corresponding controls but not different among the various drug treated groups. In the kidney, there were no differences in glomerular tuft area or tuft volume density amongst drug-treated or control animals. In contrast, tubule atrophy and interstitial fibrosis were markedly greater in CsA-treated vs RAPA-treated animals (X^2 5.00, $p<0.02$). These data suggest that whereas drug efficacy with respect to heart allograft viability is similar between CsA and RAPA, renal toxicity is significantly less in those animals receiving RAPA.

Key words: Rapamycin, Cyclosporine, Heart, Kidney, Morphology

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Introduction

Rapamycin (RAPA) is a lipophilic natural macrolide produced by Streptomyces hygroscopicus and possesses immunosuppressive activity 10-100 fold greater than cyclosporine (CsA) (Kahan et al., 1991; Kimball et al., 1991; Morris, 1992). RAPA acts differently than CsA (Metcalf and Richards, 1990; Kimball et al., 1991). It does not inhibit many early phase activation genes of lymphocytes such as IL2, IL3, IL4, IFN, INFα (Dumont et al., 1990; Metcalf and Richards, 1990, Kahan et al., 1991; Kimball et al., 1991), whereas it strongly inhibits the response of lymphocytes to these cytokines (Dumont et al., 1990).

Several studies have shown RAPA's efficacy for prevention of allograft rejection in rodents, pigs, dogs and monkeys. The organs protected include the kidney, heart, pancreas and small bowel (Collier et al., 1990; Kahan et al., 1991; Stepkowski et al., 1991, 1992). The effective dose depending on the route of administration, can be as low as 0.08 mg/kg/day (Chen et al., 1991).

Relatively few studies have investigated the toxicity of RAPA *in vivo*. Studies in the rat reported neither renal dysfunction nor histological changes in the kidneys (Kahan et al., 1991; Whiting et al., 1991; DiJoseph et al., 1992). In the dog, oral ulcers appeared by day three of treatment followed by thrombocytopenia; at autopsy, mucosal ulcers were found throughout the gut (Collier et al., 1990). Histopathological examination revealed vasculitis associated with fibrinoid necrosis.

In an earlier study from our laboratory, we compared the efficacy of RAPA vs CsA in preventing allograft rejection using a rabbit heterotopic cardiac transplant model. In this study, animals receiving RAPA exhibited excellent allograft survival, and no significant changes in hepatic or renal function were noted (Fryer et al., 1993). This species was chosen because of previous work demonstrating similarities between the rabbit, but not other species, and man in assessing CsA-induced renal side effects, notably the morphological changes in the

Table 1. Morphological assessment (mean±SD) of kidneys and heart from rabbits treated daily for 60 days with cyclosporine, vehicle or saline.

GROUP	GLOMERULAR TUFT AREA ($mm^2 \times 10^{-3}$)	GLOMERULAR TUFT VOLUME DENSITY ($mm^3 \times 10^{-2}$)	TUBULE ATROPHY*	INTERSTITIAL FIBROSIS*	HEART GRAFT REJECTION*
CSA 5 mg/kg	2.41±0.48	3.58±0.07	1.00±0.71**	1.40±0.55**	2.40±1.02**
CSA 10 mg/kg	2.33±0.42	3.66±0.19	1.25±0.50**	1.25±0.96**	1.75±1.19**
CSA 15 mg/kg	2.56±0.45	3.64±0.41	1.40±1.14**	2.25±0.96**	1.90±1.24**
Vehicle***	2.52±0.26	3.90±0.16	0	0	3.80±0.27
Saline	2.72±0.34	3.86±0.22	0	0	3.88±0.25

*: semiquantitation score 0-4; **: $p<0.05$ from controls; ***: cremophor-El.

kidney (Thliveris et al., 1991b, 1994) as well as in the concentrations and profiles of CsA metabolities (Venkataramanan et al., 1988).

In order to further assess the potential toxicity as well as efficacy of RAPA in the rabbit model, the present study was undertaken to examine histologically the native kidneys and heterotopic hearts harvested from the same animals in our previous investigations which focused on biochemical and functional parameters (Fryer et al., 1993).

Materials and methods

Cardiac allografts from smaller (1.0-2.0 kg) donors were transplanted into the necks of 50 male New Zealand white (NZW) rabbits (3.0-4.0 kg) using the technique described by Heron (1971) and modified in our laboratory (Fryer et al., 1993).

Cardiac allografts which stopped beating within 72 hours of surgery were considered technical failures and were therefore eliminated from the study. If the heart stopped beating after 72 hrs, the allograft was removed for histology and the animal continued to receive its daily intravenous injection for the duration of the study, i.e. 60 days. At the end of the study period, animals were euthanized with a fatal intravenous injection of Euthanyl. Excised cardiac allografts were processed for light microscopy and sections stained with haematoxylin and eosin (H&E) and scored semiquantitatively (by Thliveris J.A.) using the histologic grading system protocol recommended by the International Society for Heart Transplantation (Billingham et al., 1990). Kidneys were processed, sections stained with H&E and Masson, and scored semiquantitatively (by Solez K.S. and Thilivery J.A.) on a scale of $0\text{-}4^+$ (absent, minimal, mild, moderate or severe; respectively) for tubule atrophy and interstitial fibrosis as previously described (Thliveris et al., 1991a,b). Additional sections were stained with periodic acid-Schiffs (PAS) for the detection of arteriolopathy. To rule out misinterpretation of the pathological changes seen in the kidney due to the indigenous microorganisms in this instance *Encephalitozoon cuniculi*, potentially present in control and immune-suppressed animals, blood was obtained at this time of sacrifice and subjected to serologic testing, i.e., indirect immuno-fluorescent antibody and ELISA (Institut Armand-Frappier, Laval, Quebec, Canada).

Drug administration

RAPA and CsA were obtained as a gift from Wyeth-Ayerst (Priceton, NJ) and Sandoz Inc. (East Hanover, NJ) respectively. The animals were randomly divided into ten groups of 5 animals each. The group received drug or vehicle as a single daily bolus i.v. via the marginal ear vein for 60 days at the following doses: RAPA at 0.05, 0.1, 0.5 or 1.0 mg/kg/day; CsA at 5, 10 or 15 mg/kg/day; RAPA vehicle (polyethylene glycol), CsA vehicle (cremophor-El) or saline. The dose range for both drugs was selected in order to determine optimal allografts survival and minimal toxicity (Fryer et al., 1993). Drug monitoring and functional parameters i.e. body weights, creatinine clearance and liver enzymes, from these animals are detailed in our previous publication (Fryer et al., 1993).

Statistical analysis

Statistical assessment was performed using analysis of variance, X^2 and Kruskal-Wallis rank-sum test where appropriate. A p value less than 0.05 was considered significant.

Results

Morphological assessment for the transplanted hearts is presented in Tables 1, 2 and Figs. 1, 2. As can be seen, the rejection scores for heart grafts were significantly higher in saline and vehicle controls than in animals receiving either RAPA ($p<0.02$) or CsA ($p<0.03$). When comparing rejection scores amongst the group of animals receiving RAPA, there were no significant differences noted, i.e. graft survival was not dependent on concentration of drug administered. Similar results were noted among the three groups of animals treated with CsA. Moreover, there were no significant differences in graft outcome when comparing RAPA-treated versus CsA-treated animals.

Renal morphology for the native kidneys of the heart allograft recipients is also shown in Tables 1, 2 and Figs. 3, 4. As can be seen, there were no changes in glomerular tuft area or glomerular volume tuft density in

Table 2. Morphological assessment (mean±SD) of kidneys and heart from rabbits treated daily for 60 days with rapamycin or vehicle.

GROUP	GLOMERULAR TUFT AREA ($mm^2 \times 10^{-3}$)	GLOMERULAR TUFT VOLUME DENSITY ($mm^3 \times 10^{-2}$)	TUBULE ATROPHY*	INTERSTITIAL FIBROSIS*	HEART GRAFT REJECTION*
Rapa 0.05 mg/kg	2.49±0.40	3.76±0.38	0.50±0.58	0.33±0.58	2.20±0.76**
Rapa 0.10 mg/kg	2.73±0.25	4.00±0.32	0.20±0.45	0.40±0.89	1.70±0.84**
Rapa 0.5 mg/kg	3.00±0.12	3.88±0.29	0.40±0.89	0.60±1.34	1.83±1.04**
Rapa 1.0 mg/kg	2.95±0.41	3.90±0.07	0.46±0.51	0.46±0.51	1.25±0.50**
Vehicle***	2.40±0.11	3.70±0.10	0	0	3.88±0.25

*: semiquantitation score 0-4; **: $p<0.05$ from controls; ***: polyethylene glycol.

animals receiving either RAPA or CsA, compared to their corresponding controls or to each other. On the other hand all animals treated with CsA regardless of the dose exhibited the presence of tubule atrophy and interstitial fibrosis, as well as arteriolopathy, which was absent from controls. In contrast, while there was evidence of tubule atrophy and fibrosis, as well as arteriolopathy, in the kidneys from RAPA-treated animals, the severity did not reach statistical significance. This was due to the fact that most animals did not show the presence of either tubule atrophy or interstitial fibrosis, i.e. only one animal from each of the 0.05, 0.1, 0.5 and two from the 1.0 mg/kg treated groups showed changes. Moreover, when comparing the frequency of these changes between RAPA-treated and CsA-treated animals, there was a significant difference noted (X^2 5.00, $p<0.02$). Of note, three animals (one saline, one cremophor and one receiving 0.05 mg/kg RAPA) were positive for *E. cuniculi* by ELISA and were thus excluded from morphological/statistical assessment as the pathogen can induce interstitial scarring similar to that seen with CsA administration.

Discussion

CsA represents one of the more important therapeutic advances in the field of transplantation. However, its effectiveness is limited by side effects, most notably nephrotoxicity (Bennett and Normal, 1986; Myers , 1986; Thiel, 1986; Keown et al., 1987). Numerous studies, recently reviewed (Thliveris et al., 1991a) have documented this clinical entity. The functional changes

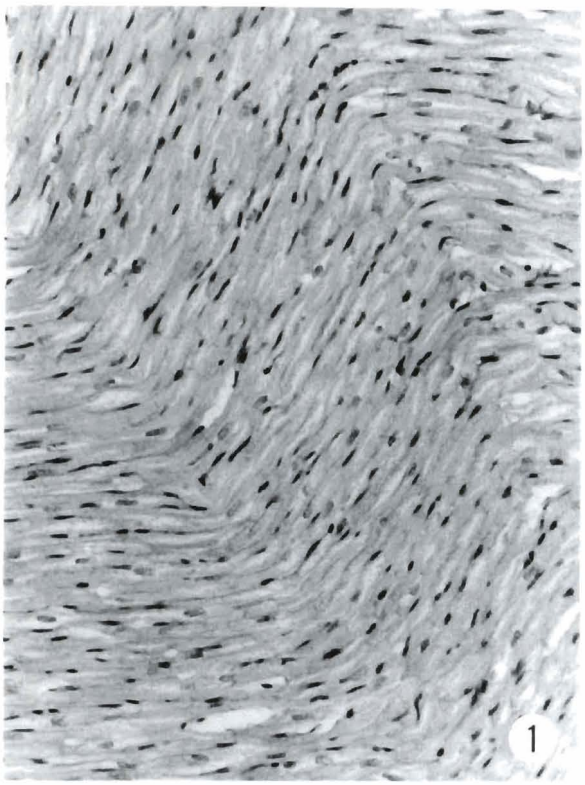

Fig. 1. Micrograph of typical myocardial fibers from a native heart of a saline control animal. H&E. x 80

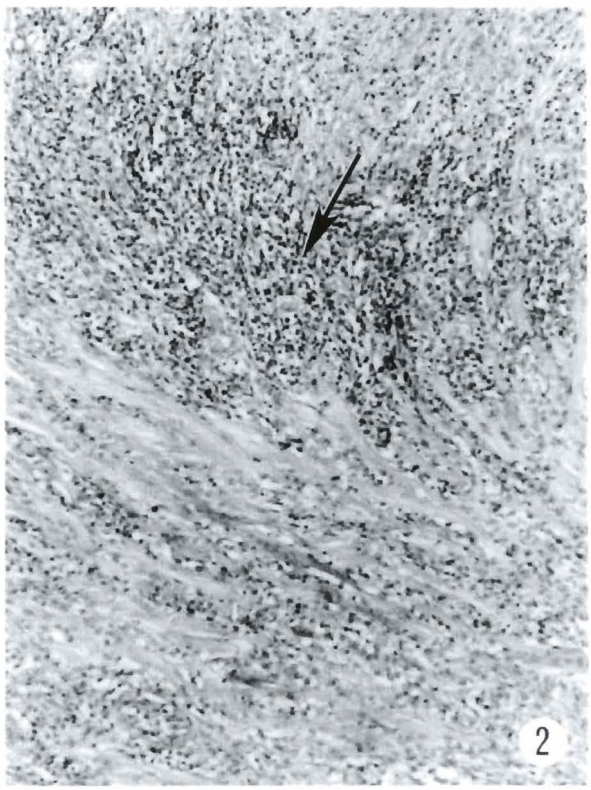

Fig. 2. Micrograph of allograft heart from a saline control animal. Note marked inflammatory infiltrate (arrows) and myocyte damage. H&E. x 50

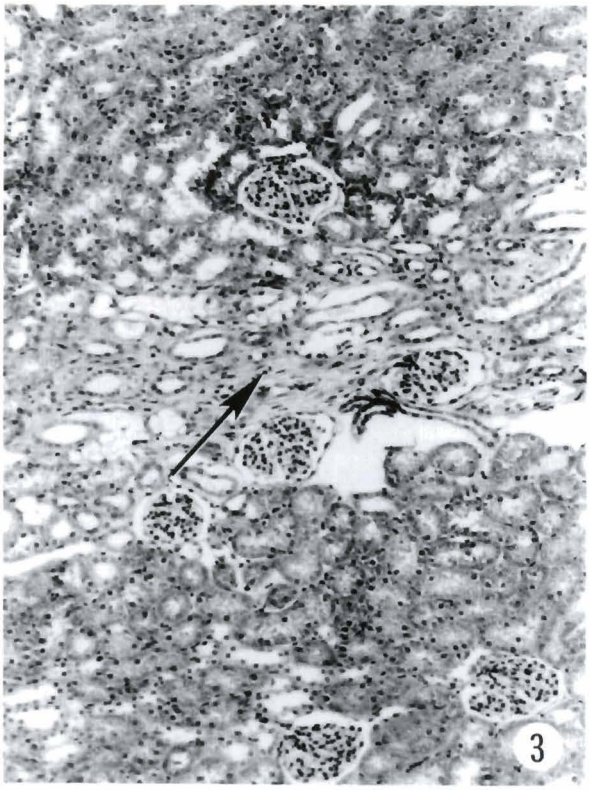

Fig. 3. Micrograph of renal cortex from a CsA-treated animal receiving a heterotopic heart transplant. Note loss of normal architecture and the presence of atrophic tubules and interstitial fibrosis (arrow). Kidneys from all animals treated with CSA showed similar changes. H&E. x 80

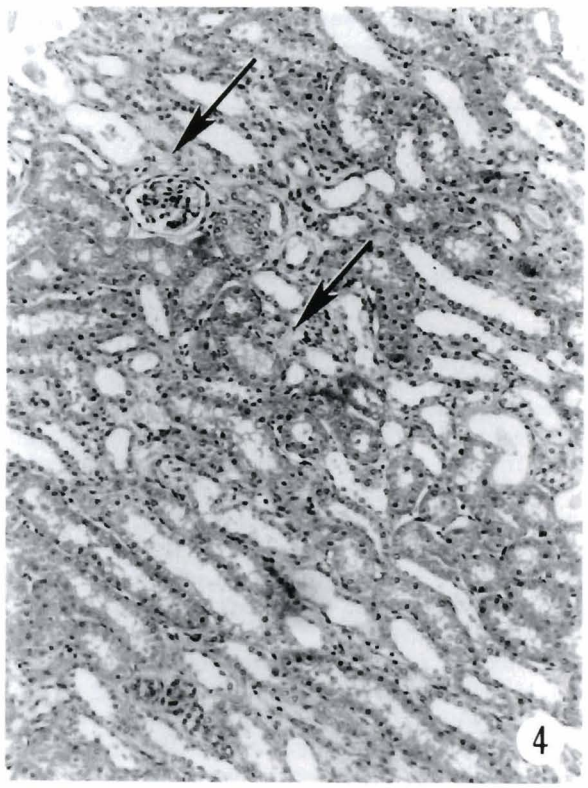

Fig. 4. Micrograph of renal cortex from a RAPA-treated animal receiving a heterotopic heart transplant. While tubule atrophy and interstitial fibrosis (arrow) were seen in this animal, most animals did not present these changes. H&E. x 80

reported are a decrease in glomerular filtration rate, renal blood flow and creatinine clearance, concurrent with an elevated serum creatinine. Morphological changes noted in the kidney are species/dose dependent and include vacuolization, micro-calcification and regeneration of proximal tubule cells, in addition to tubule atrophy, interstitial fibrosis and arteriolopathy. The latter two features, which are considered hallmarks of nephrotoxicity in man, have been noted only in the rabbit, using therapeutic dose of CsA.

The current study, while confirmational in reference to CsA, presents new information at the morphological level on RAPA, not only as an effective immunosuppressant in the rabbit but also provides some insight as to its toxicity in the kidney in this species. A previous study from our laboratory (Fryer et al., 1993) suggested that RAPA was as efficacious as CsA in preventing allograft rejection, i.e. heterotopic heart graft survival was comparable to CsA. Through concentrations of RAPA in whole blood of >10 μg/L are required for optimal graft survival while for CsA, concentrations of ~>150 μg/L were required (Fryer et al., 1993). These data are further supported by the heart graft rejection scores reported herein, i.e. histologically the heart grafts from animals treated with RAPA were similar to those from animals receiving CsA. The noteworthy difference between these two immunosuppressive agents is that RAPA was effective at a lower concentration than CsA, and that this finding may account for the lesser degree of nephrotoxicity seen on the part of RAPA. Kidneys from RAPA-treated animals showed a significantly lower frequency of structural changes compared to those from CsA-treated animals.

In summary, RAPA appears to be an effective immunosuppressant in preventing allograft rejection in the rabbit, which when administered at immunosuppressive doses results in significantly less renal side effects as compared to CsA. Moreover, the rabbit to our knowledge is the only species to show morphological changes in the kidney induced by RAPA. This finding supports our initial contention that the rabbit may be a more appropriate model to assess potential toxic effects by immunosuppressants due to its apparent sensitivity compared to other species studied. Further long term studies with RPA are warranted to more fully address this issue.

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Invited Review

Regulation of protein traffic in polarized epithelial cells

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Summary. The plasma membrane of polarized epithelial cells is divided into apical and basolateral surfaces with different compositions. Proteins can be sent directly from the trans Golgi network (TGN) to either surface, or can be sent first to one surface and then transcytosed to the other. The glycosyl phosphatidylinositol anchor is a signal for apical targeting. Signals in the cytoplasmic domain containing a ß-turn determine basolateral targeting and retrieval, and are related to other shorting signals. Transcytosed proteins, such as the polymeric immunoglobulin receptor (pIgR) are endocytosed from the basolateral surface and delivered to the apical recycling compartment underneath the apical surface. This compartment is a central sorting station, as it receives material from both surfaces and sorts them to the correct surface. Delivery to the apical surface from both the TGN and the apical recycling compartment is regulated by protein kinase A and protein kinase C, and endocytosis from the apical surface is also regulated by kinases. Transcytosis of the pIgR is additionally regulated by phosphorylation of the pIgR and by ligand binding to the pIgR. Regulation of traffic in polarized epithelial cells plays a central role in cellular homeostasis, response to external signals, and differentiation.

Key words: Golgi, Endosome, Transcytosis, Sorting signal, Recycling

Introduction

Most eukaryotic cells are spatially asymmetric or polar. Understanding how the complex three-dimensional organization of eukaryotic cells is established and maintained is a central question in cell biology. One of the simplest types of polarized cells is the epithelial cell. Epithelial cells form a layer that lines many cavities of the body, such as the lining of the digestive, respiratory and urinary systems. The plasma membrane of these cells can be generally divided into two domains, an apical or free surface that faces adjacent cells and the underlying basement membrane (reviewed in Mostov et al., 1992; Rodriguez-Boulan and Powell, 1992). The two domains are separated by tight junctions, which help to prevent mixing of the surface and provide a tight seal between cells, thereby preventing leakage across the monolayer. The two plasma membrane domains serve very different functions, and therefore have quite disparate protein and lipid compositions. For instance, in intestinal absorptive epithelia the apical surface contains enzymes involved in breaking down the food contents of the intestine, while the basolateral surface contains molecules involved in cell-cell and cell substrate adhesion. The differences between apical and basolateral surface are only one aspect to underlying polarity of the cell; however, they provide a powerful experimental window into understanding the fundamental question of cell polarity. The principles that underly polarity of epithelial cells also seem to apply to other, more difficult to study cells. For instance, neurons are perhaps the most dramatically polarized of all cells, as they have axons that can extend meters from the cell body. If a protein that is normally found in the apical cell surface of an epithelial cell is exogenously expressed in neurons, the protein is usually transported to the axonal plasma membrane. Conversely, a protein ordinarily found in the basolateral surface of epithelial cells will usually be transported to the plasma membrane of the neuronal cell body and dendrites when expressed exogeneously in neurons (reviewed in Rodriguez-Boulan and Powell, 1992).

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Sorting pathways

Epithelial cells can use a variety of mechanisms to localize proteins to the correct surface. Newly made plasma membrane proteins are made on the rough endoplasmic reticulum and the transverse the Golgi apparatus to the trans-Golgi network (TGN). There they can be packaged into vesicles that deliver them to the appropriate surface, either apical or basolateral (Fig. 1,