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E. Antoniou
A. DeRoover
Y. Nishimura
A. J. Howie
P. McMaster
M. D'Silva

Effect of RS61443 in combination with leflunomide or FK506 on rat heart allograft survival

E. Antoniou · A. DeRoover · Y. Nishimura · M. D'Silva (✉)
Transplantation Microsurgery Laboratory,
The Liver Research Laboratories,
Clinical Research Block,
Queen Elizabeth Hospital and
Medical Center, Edgbaston,
Birmingham B15 2TH, UK
Fax + 44 1216272497

A. J. Howie
Department of Pathology,
University of Birmingham, Edgbaston,
Birmingham B15 2TH, UK

P. McMaster
The Liver and Hepatobiliary Unit,
Queen Elizabeth Hospital and
Medical Center, Birmingham, UK

Abstract Mycophenolate Mofetil (RS61443) is a potent inhibitor of de novo purine synthesis and lymphocyte proliferation. It is known to prevent ongoing rejection and even reverse established rejection, alone and in combination with, cyclosporin. We investigated whether RS61443 in combination with leflunomide (Lef) or FK506 (FK) could prolong allograft survival in a rat heart model, since combination therapy might help to overcome drug toxicity. Abdominal heart grafting was performed from DA to LEW rats (250 g) and RS61443, 10 mg/kg or 30 mg/kg monotherapy or combination treatment (RS 10 mg/kg with Lef 5 mg/kg or FK 0.5 mg/kg) was begun orally at transplantation and continued daily until the ninth posttransplant (post-Tx) day. Ventricular motion was graded daily and rejection was defined as lack of contractions, confirmed by histology. Results were analysed using non-parametric tests. A two-tail P value < 0.05 was considered significant. RS at 10 mg/kg was ineffective and all grafts were rejected under immunosuppression between 5 and 6 post-Tx day, whereas RS at 30 mg/kg was immunosuppressive for as long as it was given. The combinations of RS at 10 mg/kg with either Lef at 5 mg/kg or FK at 0.5 mg/kg were immunosuppressive in the majority of cases, for as long as they were given. However, the combination of RS at 10 mg/kg with FK at 0.5 mg/kg was attendant with graft vein anastomosis rupture (3/5). The combination of RS with Lef was clinically therapeutic for as long as it was given; grafts were rejected 3–4 days after withdrawing immunosuppression. The combination of RS with FK resulted in over-immunosuppression, leading to graft vein anastomosis non-healing and rupture within 5 days of grafting; histology demonstrated evidence of bacterial infection with complete destruction of the vein wall. These data suggest that the combination of RS with Lef or FK in subtherapeutic doses might be a potentially promising strategy for combination therapy in solid organ transplantation.

Key words Immunosuppression · Rejection · Combination therapy · RS61443 · Heterotopic heart transplantation

Introduction

Mycophenolate Mofetil (RS61443) is a semisynthetic prodrug of mycophenolic acid (MPA), which selectively, non-competitevely and reversibly inhibits the de novo pathway for the purine synthesis required for T and B cells proliferative responses to antigens and mitogens [5]. This drug has been found to have antifungal, antiviral and antibacterial properties, and it has also been tested clinically in the treatment of psoriasis [6]. RS61443

Table 1 Heart allograft survival under monotherapy or combination therapy immunosuppression (*RS* RS61443, *Lef* leflunomide, *FK* FK506)

Group	Treatment (days 0–9 post-transplantation)	Survival (days), median (range)	*P*	Comments	Graft histology
1 ($n = 6$)	–	5 (4–5)		–	Severe AR 5/5
2 ($n = 4$)	RS 10 mg/kg	5.5 (5–6)	0.14	–	Severe AR 4/4
3 ($n = 5$)	RS 30 mg/kg	13 (13–14)	0.009	–	Severe AR 5/5
4 ($n = 5$)	RS 10 mg/kg + Lef 5 mg/kg	13 (13–14)	0.009	–	Severe AR 5/5
5 ($n = 5$)	RS 10 mg/kg + FK 0.5 mg/kg	17.5 (14–21)[a]	0.05	3/5 vein anastomosis rupture	Severe AR 2/5

[a] Excluding grafts with ruptured vein anastomoses

has shown efficacy in several models of organ transplantation, preventing ongoing rejection and even reversing established rejection, alone and in combination with, cyclosporin [3, 4].

We investigated whether RS61443 (RS) in combination with leflunomide (Lef) or FK506 (FK) could prolong allograft survival in a rat heart model, since combination therapy might help to overcome drug toxicity.

Materials and methods

Heterotopic intraabdominal heart transplantation was performed, according to a previously reported technique [1], using adult male DA(RT1[a]) as donors and Lewis-RT1[l] (LEW) rats as recipients, weighing 200–250 g. The subjects were commercially obtained from Charles River, UK and cared for humanely during the course of the study, according to prevailing Home Office Guidelines in the UK. RS and Lef were suspended in 1 % carboxymethyl cellulose (CMC). FK was dissolved in normal saline.

RS monotherapy or combination therapy was administered orally daily for 10 days, starting the day of grafting until the ninth posttransplant day. Ventricular motion was graded daily and rejection was defined as lack of contractions, confirmed by histology. Recipients with rejected grafts or adverse clinical signs were euthanised and tissues analysed histologically. Results are expressed as median survival days and analysed using non-parametric tests. A two-tail P value < 0.05 was considered significant.

Results

Table 1 shows the results of this study using a fully major histocompatibility complex (MHC) incompatible strain combination (DA to LEW), in which freshly harvested DA hearts were transplanted intraabdominally to LEW recipients. In the control group, graft rejection was complete between 4 and 5 days postoperatively. The median survival time was 5 days. RS at 10 mg/kg was ineffective and all grafts were rejected under immunosuppression between 5 and 6 days posttransplantation, whereas RS at 30 mg/kg was found to be immunosuppressive for as long as it was given, prolonging graft survival for a period of 4–5 days after termination of immunosuppression. The median survival time in the first group (RS 10 mg/kg) was 5.5 days, not significantly different from controls. In the second group (RS 30 mg/kg) the median survival time was 13 days (range 13–14 days), significantly different from controls.

The combinations of RS at 10 mg/kg with either Lef at 5 mg/kg or FK at 0.5 mg/kg were immunosuppressive in the majority of cases, for as long as they were given. However, the combination of RS at 10 mg/kg with FK at 0.5 mg/kg led to graft vein anastomosis rupture (3/5). The median survival time in the first combination (RS + Lef) was the same as the RS at 30 mg/kg group. Apart from the rats with the ruptured vein anastomoses in the second combination group (RS + FK), the grafts of the remainder rats achieved a prolonged survival of 14–21 days (median survival time 17.5 days), significantly different from controls.

Discussion

In this study, we have used RS monotherapy (10 and 30 mg/kg) and RS in combination with Lef or FK in an effort to prolong heterotopic heart allograft survival in the rat. RS at 10 mg/kg was found to be subtherapeutic, all grafts rejecting under treatment, whereas 30-mg/kg dose prolonged allograft survival 8 days beyond DA × LEW controls. Moreover, combination therapy of RS at 10 mg/kg with Lef at 5 mg/kg was clinically therapeutic for as long as it was given; grafts were rejected 3–4 days after withdrawing immunosuppression. On the other hand, the combination of RS at 10 mg/kg with FK at 0.5 mg/kg resulted in over-immunosuppression, leading to graft pulmonary vein anastomotic non-healing and rupture within 5 days of grafting. Histology demonstrated evidence of bacterial infection with complete destruction of the vein wall. However, 2/5 grafts survived an additional 14 and 21 days after discontinuing treatment. Previous studies [2] have demonstrated that long-term heart allograft survival was achieved with RS at 20 mg/kg per day and brequinar at 3 or 6 mg/kg, 3 times per week. Our data, however, suggest

that the combination of RS with Lef or FK in subtherapeutic doses may be a potentially promising strategy for combination therapy in solid organ transplantation.

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References

1. D'Silva M, Candinas D, Achilleos O, et al (1995) Transplantation 60: 1–8
2. Kawamura T, Hullett DA, Suzuki Y, et al (1993) Transplantation 55: 691–695
3. Knechtle SJ, Wang J, Burlingham WJ, et al (1992) Transplantation 53: 699–701
4. Laskow DA, Deierhoi MH, Hudson SL, et al (1994) Transplantation 57: 640–643
5. Morris ER, Hoyt EG, Murphy MP, et al (1990) Transplant Proc 22: 1659–1662
6. Platz KP, Sollinger HW, Hullett DA, et al (1991) Transplantation 51: 27–31