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Prevention of cardiac allograft rejection by FK506 and rapamycin: assessment by histology and nuclear magnetic resonance

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Abstract. We assessed the effect of FK506 and rapamycin (RPM) in a heterotopic abdominal rat heart transplant model using a major histocompatibility mismatch (DA to LEW). The end-point of our study was the histologic grading of rejection (Stanford) and ^{31}P magnetic resonance spectroscopy (MRS) at 1 week after transplantation. Two dosages of FK506 (2.0 and 8.0 mg/kg per os daily) and RPM (1.5 and 6.0 mg/kg intraperitoneally daily) were compared in allografts without and with cyclosporine (12.5 mg/kg per os daily) treatment. The results show: Weak heartbeat and full rejection at day 5 in all untreated allografts; severe rejection in groups on a low dose of FK506 and RPM; mild rejection in both high dose groups comparable to the results of the hearts treated with cyclosporine; MRS does not allow differentiation between no or mild forms of rejection. Energy-rich phosphates are near normal in the high dosage immunosuppression groups but show a significant reduction in the low dosage groups. We conclude that all three tested drugs can reduce the degree of rejection from severe (untreated allografts) to mild if given in an adequate dosage. MRS correlates well with the degree of histologic rejection but permits only the diagnosis of moderate or severe rejection.

Key words: Immunosuppression – Cardiac transplantation – Rejection – Magnetic resonance spectroscopy

Several serious side-effects have been reported in organ transplantations with various immunosuppressive regimes [1]. The need for life-long drug administration leads to irreversible adverse drug effects including conditions such as hypertension and renal failure or increased incidence of malignancies and consequences from corticosteroid treatment [2]. New immunosuppressive medication with fewer side-effects for long-term treatment or for use as a rescue drug for acute rejection have been proposed, such as FK506 [3, 4] and rapamycin (RPM) [5, 6].

We tested the drug efficacy and toxicity of FK506 and rapamycin in two different dosages and compared their immunosuppressive action with that of cyclosporine (CyA) in the heterotopic rat allotransplant model using a major histocompatibility mismatch (DA to LEW). Alterations of energy-rich phosphate compounds (^{31}P) were assessed in vivo by magnetic resonance spectroscopy (MRS) 1 week after transplantation, followed by euthanasia and histology evaluation.

Method

A major histocompatibility mismatch was used, DA/HAN (AV1) rats serving as donors (10–12 weeks old/200 g) and Lewis – LEW/HAN (RT1) rats as recipients (8–10 weeks old/250 g Hannover strains). For the group of isografts, Lewis rats served as donors and recipients. The classic heterotopic abdominal rat heart transplant model described by Ono and Lindsey was applied [7]. Donor hearts were flushed with ice-cold St. Thomas cardioplegic solution. Warm ischemia ranged between 30 and 35 min. Only well functioning hearts after the completion of transplantation were assigned in turn to a treatment group.

The following immunosuppressive drugs were used:
FK506 (Fujisawa Pharmaceuticals Ltd., Osaka, Japan) was given per os by gavage at a dose of 2 mg/kg or (mg/kg pure drug). Rapamycin (RPM; Wyeth-Ayerst Research, Princeton, N.J.) was given by intraperitoneal injection at a dose of 1.5 mg/kg or 6 mg/kg. Both drugs were suspended after micronisation in carboxymethyl cellulose (CMC 0.2%, high viscosity) and further agitated in an ultrasonic bath before use. CyA (Sandoz Pharmaceuticals, Basel, Switzerland) was mixed in olive oil and given by gavage at a dose of 12.5 mg/kg. All drugs were given daily from postoperative day 1 until euthanasia (day 6 or 7).

Table 1 lists the different groups according to the immunosuppressive drug used and the respective dosage. All animals were assessed daily for their general condition, and the heartbeat of the abdominally transplanted heart was noted.

At the end point of our study (1 week), the animals were reanesthetised, and a median laparotomy was performed to allow in vivo MRS measurements. Each acquisition consisted of 512 FIDs (repetition time 2.0–2.4 s, spectral width ± 2000 Hz, resolution 4096 points) performed on a 2-T General Electric-CSI wide bore

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Table 1. Groups and number of animals listed according to immunosuppression and dosage after transplantation

Groups	POD	Dosage (mg/kg)	Number
Isografts (LEW-LEW)	10.5	–	6
Allografts (DA-LEW)	4.8	–	6
+ CyA	7.6	12.5	6
+ FK506 (low)	7.0	2	5
+ FK506 (high)	6.3	8	6
+ RPM (low)	6.0	1.5	5
+ RPM (high)	5.8	6	6

POD, mean postoperative day of examination; CyA, cyclosporine; RPM, rapamycin

magnet with a double tuned, 15-mm surface coil placed around the apex of the transplanted heart. Quantification of the spectroscopy scan was carried out using a Lorentzian curve-fitting procedure. MRS results are expressed as ratios between energy-rich phosphates such as phosphocreatine (PCr) or adenosine triphosphate (β-ATP) and inorganic phosphate (Pi). After the completion of MRS, the animals were euthanised and the hearts excised for histology evaluation (hematoxylin & eosin and Mason's trichrome stains). Five transverse sections of each heart were reviewed by two independent pathologists. The histologic evaluation of rejection was performed according to the classic Stanford grading system [8]: 0 = no signs of rejection, 1 = mild rejection with focal perivascular infiltrates, 2 = moderate rejection with additional focal myocyte necrosis, 3 = severe rejection with the above and focal hemorrhage.

Results

The efficacy of the tested immunosuppressive drugs was assessed by MRS alterations and the histologic degree of rejection at the end-point of our study. Table 2 summarises the results according to the different groups.

Graft survival and histology

All isografts survived well and showed a strong heartbeat up to euthanasia. The hearts did not reveal any signs of rejection histologically.

All untreated allografts survived well, but the transplanted heart showed a weak heartbeat or ceased to beat between days 5 and 6 and showed severe rejection on all histologic sections (grade 3).

Allografts treated with cyclosporine showed a strong heartbeat on day 7, and the animals tolerated the dosage of 12.5 mg/kg well. On histology the mean rejection score was 0.8, ranging from 0.4 to 1.0.

Animals treated with low dose FK506 had no adverse reaction to the drug but demonstrated a decreased heartbeat strength, with 1 heart out of 5 showing no function at day 7. The mean score of rejection was 2.4 (moderate to severe), ranging from 2 to 3.

In the high dose FK506 group all grafts showed a strong heartbeat, and histology revealed a mild degree of rejection (1.2) ranging from 0.5 to 1.5.

In the low dose RPM group all transplanted hearts either showed poor heartbeats or had ceased beating by 6 days after transplantation. Histologic analysis showed a mean grading of rejection of 2.8, ranging from 2.5 to 3.0.

In the high dose RPM group the heartbeats were strong, and the grafts looked normal. On histology the mean grading was 1.1 (mild rejection) with a range from 0.5 to 1.5.

Adverse reactions to immunosuppression

Except for 2 animals (1 FK506 high dose and 1 RPM high dose) showing symptoms consistent with drug toxicity (weight loss and lethargy despite a well-functioning transplanted heart), no lesion patterns indicative of drug toxicity were found upon histology examination of the animals' own heart, lungs, liver, kidneys, and pancreas. Only 1 animal in the FK506 high dose group receiving a daily dose of 10.5 mg/kg showed swollen kidneys and severe edema of most organs, probably a sign of drug toxicity.

Magnetic resonance spectroscopy

The assessment of energy metabolism by MRS is represented in Fig. 1 (spectrum from one animal) and by groups in Table 2; results are expressed as ratios between energy-rich phosphates and inorganic phosphate such as PCr/Pi or β-ATP/Pi. In isografts and in allografts with adequate immunosuppressive therapy, that is high doses of CyA, FK506, or RPM, no significant alterations in the phosphate metabolism could be detected (mild rejection on histology).

On the other hand, untreated allografts or allografts with insufficient immunosuppression (moderate to severe rejection) such as FK506 low dose or RPM low dose, the PCr/Pi and β-ATP/Pi ratios decreased significantly ($P < 0.05$) by 50% or more (Table 2).

Discussion

In our study we were able to show that relatively high doses of the three tested immunosuppressive drugs, i.e., CyA, FK506, and RPM, controlled the severe rejection that occurred in untreated allografts. Comparing our results with those published by R. Morris at Stanford

Table 2. Magnetic resonance spectroscopy (MRS) phosphate ratios and histologic degree of rejection according to groups

Groups	PCr/Pi	β-ATP/Pi	Rejection[a]
Isografts (LEW-LEW)	2.4 ± 0.9	1.1 ± 0.3	0
Allografts (DA-LEW)	1.6 ± 0.7	0.7 ± 0.5	3.0
+ CyA	2.3 ± 0.8	1.0 ± 0.2	0.8 ± 0.5
+ FK506 (low)	1.1 ± 0.1*	0.6 ± 0.2*	2.4 ± 0.8
+ FK506 (high)	2.3 ± 0.7	0.8 ± 0.4	1.2 ± 0.4
+ RPM (low)	1.0 ± 0.8*	0.5 ± 0.2*	0.8 ± 1.2
+ RPM (high)	1.9 ± 0.2	1.0 ± 0.1	1.1 ± 0.4

MRS result is expressed as ratios between energy-rich phosphates such as phosphocreatine (PCr) or adenosine triphosphate (β-ATP) and inorganic phosphate (Pi)

[a] Degree of rejection is based on the classic Stanford grading [ST] (0–3)

Results are expressed as mean values ± 1 SD. Significance by Student's *t*-test; * $P < 0.05$

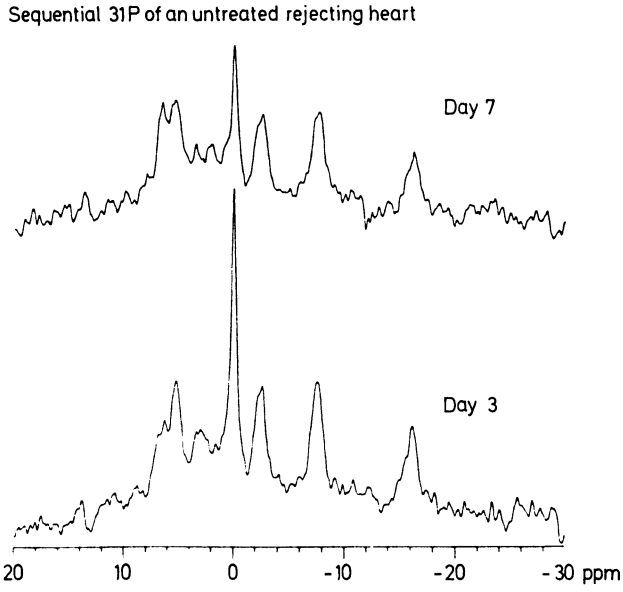

Fig. 1. Sequential ^{31}P MRS of an untreated rejecting heart. Peaks from left to right: phosphomonoester *(PME)*, inorganic phosphate *(Pi)*, phosphodiester *(PDE)*, phosphocreatine *(PCr)*, adenosine triphosphate (γ, α, β-*ATP*). Represented are a near normal ^{31}P spectrum 3 days after heterotopic transplantation *(lower panel)* and a severely altered spectrum of the same animal after 7 days *(upper panel)* with decreased PCr and ATP and increased PME and Pi (untreated allograft with severe histological rejection)

University using the same drug, it is noteworthy that he obtained a good level of immunosuppression with lower doses than we did [5, 9]. One possible explanation might be the different rat strains used. According to the literature and our own experience, the combination of DA to LEW rats seems to count among the strongest major histocompatibility mismatch models as reflected in our series by the cessation of heartbeat and full histologic rejection at day 5 to 6 in untreated allografts [10].

We purposefully set the end-point of our study at 1 week (most severe immune response) to assess the efficacy of immunosuppression [11]. As seen in Table 1, the mean postoperative day of the histologic examination varied only between days 6 and 7 for the treated allografts, whereas in the untreated allografts it was on average at 4.8 days due to the rapid deterioration of the graft (weak heartbeat). In contrast, isografts were doing well even at 10 days since in this group no immunological reaction occurred.

MRS findings correlated well with histologic grading in moderate or severe rejection, but MRS was not sensitive enough to differentiate between no and mild rejection. This might be due to the sparse and focal myocardial involvement seen in mild rejection.

The relatively good MRS results obtained in the untreated allografts are biased due to the early postoperative MRS assessment (4.8 versus 7 days in the other groups) necessary to perform in vivo MRS on beating hearts. Our MRS findings are less sensitive but comparable with the ones reported by Canby et al. [12] and Fraser et al. [13].

In conclusion, high doses of CyA, FK506, and RPM achieved adequate immunosuppression even in our strong major histocompatibility mismatch model, since none of the hearts showed more than mild (clinically not requiring treatment) or mild to moderate rejection at 1 week after transplantation. However, further investigations are needed to assess the possible toxic effects of FK506 and RPM at high dose levels. MRS findings confirm alterations in the phosphorous metabolism in moderately to severely rejecting hearts.

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