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The immunomodulatory effects of a novel agent, leflunomide, in rat cardiac allotransplantation

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Abstract We assessed the immunomodulatory effect of leflunomide (LEF) in a heterotopic abdominal model of rat heart transplantation using a major histocompatibility mismatch (DA X LEW). The endpoint of this study was cardiac rejection assessed by abdominal palpation of the ventricular impulse and confirmed by laparotomy and histology. In this study, LF was investigated using four dosages (5, 10, 20 and 30 mg/kg per day orally) against cyclosporine (CsA) (15 mg/kg per day orally) and FK 506 (1 mg/kg per day orally). The ability of LEF to prevent rejection and reverse ongoing acute rejection was assessed. The results showed that untreated hearts were fully rejected by day 5 and that LEF at 5 mg/kg was significantly better than any other dose tested, was superior to FK 1 mg/kg, and was as effective as CsA 15 mg/kg in preventing rejection after a short course of treatment. After a longer course, 10 and 20 mg/kg LEF proved more effective than 5 mg/kg in controlling rejection and as efficacious as 1 mg/kg FK and 15 mg/kg CsA. In the control of ongoing established early rejection. LEF proved to be equally effective, even at lower doses (5 mg/kg), to CsA 15 mg/kg and FK 1 mg/kg. In the control of ongoing established late rejection, 20 mg/kg LEF proved to be superior to 10 mg/kg LEF and 15 mg/kg CsA, and was as effective as FK 1 mg/kg. However, when higher doses of CsA (25 mg/kg) and FK (2 mg/kg) were tested against 20 mg/kg LEF in this mode of rescue, LEF proved as effective as CsA and superior to FK. In the assessment of drug toxicity using body weight as a parameter, 20 mg/kg LEF proved safer than any other LEF dose tested, and safer than 15 mg/kg CsA and 1 mg/kg FK in both short- and long term administration. We conclude that LEF is a relatively safe and potent immunosuppressant with promising clinical potential.

Key words Leflunomide
Immunosuppression · Cyclosporine
FK 506

Introduction

In the light of the adverse effects encountered with current cyclosporine-based immunosuppression protocols, newer immunosuppressants with fewer side effects in long-term administration provide an attractive and alternative option for the future management of transplanted patients on life-long immunosuppression. Leflunomide (LEF) is one such novel agent that has been proven effective in animal models of autoimmune disease [1] and in experimental organ transplantation [2, 4]. The aims of this study were to examine the efficacy and toxicity of LEF and to compare its immunosuppressive action with that of cyclosporine (CsA) and FK 506 in the heterotopic rat heart allotransplant model (DA × LEW).

Materials and methods

Inbred male DA (RT1^a) and LEW (RT1^l) rats, weighing 200–250 g were used as donors and recipients, respectively, for heterotopic abdominal heart transplantation. For the syngeneic controls, LEW rats served as donors and recipients. Cardiac transplantation was carried out using the technique of Ono and Lindsey [3] with modification under enflurane anaesthesia. The following immunosuppressants were used. LEF (a kind gift from Hoechst, Wiesbaden, Germany) was given after suspending it in 1% carboxymethyl cellulose (CMC). CsA (Sandoz Pharmaceuticals, Basel, Switzerland) was also mixed in 1% CMC. FK 506 (Fujisawa Pharmaceuticals, Munich, Germany) was dissolved in normal saline. All drugs were given orally daily from the day of transplantation until the 10th or 30th day post-transplant, depending on the schedule. In the rescue treatment mode, drugs were given from day 2 until day 6 or day 4 until day 6 post-transplant. Survival data were analysed using non-parametric tests and body-weight data were subjected to linear regression analysis. A P-value < 0.05 was set as statistically significant.

Results

Graft survival (Table 1)

In the vehicle-treated control group, the graft survived for a median of 5 days. Syngeneic LEW recipients survived indefinitely (median > 100 days). The analyses proved that graft survival seen for the different LEF dosages tested was not dose dependent. LEF 5 mg/kg was significantly better than any other dose tested. It was also superior to 1 mg/kg FK and as effective as 15 mg/kg CsA in prolonging graft survival. Following a 30-day course of immunosuppression, LEF exhibited a statistically significant dose-dependent increase in median graft survival time. However, 20 and 30 mg/kg LEF proved more effective than 5 mg/kg LEF but comparable to 15 mg/kg CsA and 1 mg/kg FK. LEF at a lower dose (5 mg/kg) was as effective as 10 mg/kg in controlling ongoing acute early rejection, and as effective as 15 mg/kg CsA and 1 mg/kg FK. However, 20 mg/kg LEF proved more effective than 10 mg/kg LEF in controlling ongoing acute late rejection, and, was also found to be superior to 15 mg/kg CsA but as effective as 1 mg/kg FK as a late rescue agent. This order changed when higher doses of CsA (25 mg/kg) and FK (2 mg/kg) were tested against 20 mg/kg LEF. The results showed that 20 mg/kg LEF was as effective as CsA and superior to FK.

Drug toxicity (Fig. 1 and 2)

Overall, in both short- and long-term administration, and 20 mg/kg LEF proved safer in restoring the preoperative

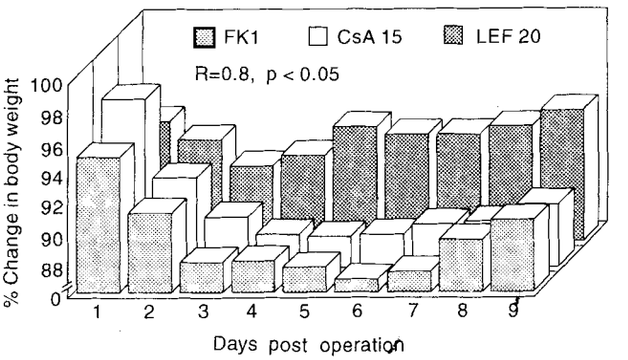

Fig. 1 Percentage change in body weight after a 10-day course of leflunomide, CsA and FK 506 treatment

Table 1 Survival data (days) in (DA × LEW) recipients under leflunomide, CsA and FK 506 Immunosuppression[a]

[a] Statistically significant results are discussed in the text
[b] Drugs given from day 2 to day 6
[c] Drugs given from day 4 to day 6

Group	10-days course Median (range)	30-day course Median (range)	Early rescue[b] Median (range)	Late rescue[c] Median (range)
Control	5 (4–5)	5 (4–5)	5 (4–5)	5 (4–5)
LEF 5 mg/kg	21 (20–29)	29 (24–33)	10 (10–11)	–
LEF 10 mg/kg	14 (14–15)	35 (32–42)	10.5 (9–12)	10 (9–10)
LEF 20 mg/kg	13 (13–14)	44 (35–57)	–	13 (10–17)
LEF 30 mg/kg	18 (14–19)	–	–	–
CsA 15 mg/kg	18 (13–35)	38 (37–44)	11 (8–12)	8 (7–10)
FK 1 mg/kg	12 (6–20)	45 (41–48)	11 (10–16)	6 (6–26)

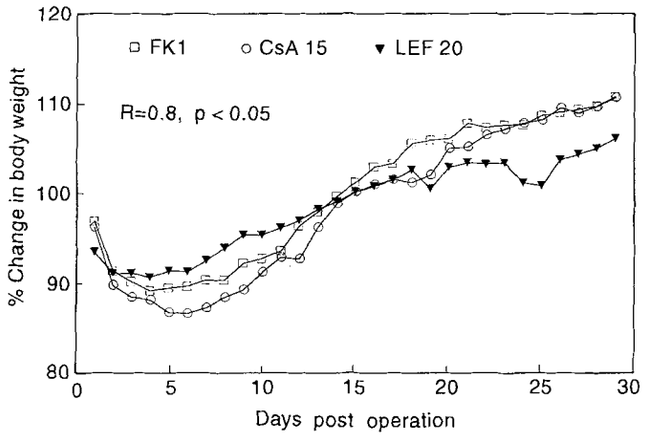

Fig. 2 Percentage change in body weight after a 30-day course of leflunomide, CsA and FK 506 treatment

body weight than any other dose examined. Additionally, these doses were also found to be safer than either CsA or FK.

Discussion

LEF is an isoxazole derivative which has previously been shown to be effective in autoimmune diseases and to prevent acute rejection of skin [2], cardiac [4] and kidney [2] grafts in rats. The results of our study suggest that LEF has an immunomodulatory effect in rat recipients of cardiac grafts providing it is administered continuously. The immunosuppressive effect terminated a short time after discontinuation of the drug, at which time graft histology for the various drug groups was not vastly different, all showing complete rejection with variations in the quantity of loss of healthy myocytes, infiltrates and oedema. In our study, we did not encounter indefinite graft survival. However, the immunosuppressive effect was shown to be similar to CsA and FK 506 in both prevention of rejection and rescue treatment modes.

In the assessment of LEF's ability as a potent rescue agent, we intentionally discontinued treatment after 5 and 3 days of drug administration for early and late rescue effects, respectively. Under these conditions, LEF proved to be an effective rescue agent in comparison with CsA and FK 506.

We also conclusively demonstrated that oral administration of LEF (at 5 and 20 mg/kg per day) was not complicated by gastrointestinal toxicity or weight loss, in contrast to therapeutic doses of CsA and FK 506. We conclude that LEF is a relatively safe and potent immunosuppressant with promising clinical potential.

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