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LF 08–0299 In the prophylaxis and treatment of chronic rejection in a rat aortic allograft model

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Abstract Chronic rejection is the major cause of late kidney allograft failure. We evaluated the efficacy of LF 08–299 (LF), an analogue of 15-deoxyspergualin, in a rat aortic allograft model of chronic rejection. BN aortic allografts were transplanted to Lew recipients. LF was administered at a dose of 6 mg/kg and 2.5 mg/kg on days 0–20 and 6 mg/kg on days 60–90. CyA was used at a dose of 5 mg/kg on days 0–20. Untreated isografts and allografts were used as controls. Histological changes and immunohistochemistry were monitored sequentially at 8, 12, 16 and 20 weeks. There were no differences in intimal proliferation between LF-treated allografts and untreated or CyA-treated controls. Only a tendency in adventitial infiltration reduction was seen in LF-treated animals. We found a significantly less pronounced reduction in media diameter in LF-treated animals. We concluded that LF 08–0299 is only able to reverse reduction in media thickness in aortic allografts, but not intimal proliferation in this model of chronic rejection.

Key words LF 08–299 · Chronic rejection · Immunosuppression aortic allograft model

Introduction

Despite the advances in immunosuppressive therapy over past decades resulting in improved early allograft survival, chronic rejection has remained a major cause of graft loss in renal, heart and lung recipients [1]. Alloantigen-dependent and -independent factors play an important role in the pathophysiology of chronic graft failure [1, 2]. The most significant histopathologic pattern in chronically rejected organs is diffuse concentric intimal proliferation of the muscular arteries resulting in obliterative lesions. The same arteriopathy has been described in aortic allografts transplanted in major histocompatibility complex disparate rats [3, 4].

LF-08–0299, an analogue of 15-deoxyspergualin, is a new potent immunosuppressive agent, capable of inducing permanent acceptance of a fully mismatched heart allograft in rats and preventing graft versus host disease [5, 6]. The aim of the present study was to evaluate the efficacy of LF-08–0299 in the prophylaxis and treatment of chronic rejection in a rat aortic allograft model.

Material and methods

Abdominal aortic segments of BN ($RT1^n$) rats were transplanted (end-to-end) to LEW ($RT1^l$) recipients using non-sterile microsurgical technigues (Ethilon 10/0). LF-08–0299 was administred at a dose 6 mg/kg body weight (b.w.; group E) and 2.5 mg/kg b.w.

Table 1 Immunosuppressive scheme and number of animals in different groups

Group	Transplantation	Drug	Dose mg/b.w.	Schedule (days)	Number of animals			
					2 months	3 months	4 months	5 months
A	Syn control	None	–	–	2	3	3	3
B	Allo control	None	–	–	3	3	3	3
C	Allo	CyA	5 mg	0–20	6	6	5	3
D	Allo	LF 08-299	2.5 mg	0–20	6	6	6	–
E	Allo	LF 08-299	6.0 mg	0–20	5	6	6	–
F	Allo	LF 08-299	6.0 mg	60–80	–	6	6	6

Table 2 Intimal proliferation (intima thickness) of aortic segment allografts and isografts

	2 months (μm)	3 months (μm)	4 months (μm)	5 months (μm)
Syn	35.95 20.65	17.43 14.98	3.30 0.70	9.45 5.34
Allo	68.77 14.54	61.06 26.07	77.50 10.94	82.17 9.93
CyA	77.18 26.11	67.58 17.78	76.32 26.28	76.93 5.97
LF 2,5 Prophyl.	69.43 20.95	70.78 26.21	85.07 6.87	–
LF 6 Prophyl.	92.83 5.92	84.38 14.97	90.23 22.80	–
LF 6 Treatment	–	76.42 23.00	82.50 12.35	86.57 15.35

Significant differences between allografts and isografts not shown

Table 3 Thickness of media in aortic segment allografts and isografts

	2 months (μm)	3 months (μm)	4 months (μm)	5 months (μm)
Syn	73.95 5.6	76.43 14.98	78.87 6.5	73.95 3.46
Allo	50.10 3.76	54.23 13.7	41.07* 2.89	46.00 1.39
CyA	71.10 6.11	52.58 13.91	50.10 9.19	47.67 3.78
LF 2,5 Prophyl.	55.88 8.52	48.48 5.33	51.53* 7.55	–
LF 6 Prophyl.	70.10 3.66	52.65 8.03	55.72** 8.35	–
LF 6 Treatment	–	49.32 6.25	45.60 5.13	48.82 6.86

Kruskal-Wallis analysis: * $P < 0.05$; ** $P < 0.1$

(group D) intraperitoneally on days 0–20 (prophylaxis). In group F animals, LF-08–0299, 6 mg/kg b.w., was used on days 60–90 after transplantation (treatment). Cyclosporin A (CyA; group C) at a dose of 5 mg/kg b.w. was administred intraperitoneally on days 0–20 (treatment control). Untreated isografted (A) and allografted (B) animals served as controls. The study design and number of animals are summarised in Table 1.

Histological changes, intimal proliferation (thickness) and cellularity, adventitial infiltration, media necrosis, cellularity and thickness were monitored sequentially by light microscopy and immunohistochemistry in treated rats and untreated controls at 8, 12, 16 and 20 weeks when the recipients were killed. The averages of individual grade values (0–3) in all animal groups and posttransplant intervals were used to calculate the semiquantitative mean index (SMI). Intimal thickness (from endothelial surface to the inner border of media) and media thickness (mean of the maximal and minimal distance between the internal and external elastic membranes) were measured with a calibrated eyepiece graticule at six angular intervals (60° each), and the mean values were calculated for individual aortas and animal groups. Statistical analyses were performed using Kruskal-Wallis analysis.

Results

Aortic segment transplantation was performed in 99 animals. Three animals died during the trial. In allogeneic control animals, intimal proliferation, media diameter reduction and adventitial infiltration were the main markers of damage. The histological pattern in the aortic segments of animals treated with CyA at 5 mg/kg b.w. were similar to those seen in allogeneic controls, there were no significant differences in the followed markers.

Intimal proliferation as the main marker of chronic allograft vasculopathy increased over time after transplantation in all allografts (significantly compared to syngeneic controls, $P < 0.01$) There were no differences in intimal proliferation between LF 08–299 treated allografts and untreated or CyA-treated controls. (Table 2). We only found a tendency towards reduced adventitial infiltration in the aortic walls of animals treated with LF 08–299. There were no differences in macrophage ($ED1^+$ cells), $CD4^+$ or $CD8^+$ lymphocyte infiltration of treated aortic allografts and their untreated controls.

Media necrosis, evaluated with a semiquantative method, was present in all aortic allografts, and no reduction was found in LF 08–299 or CyA-treated animals. Nevertheless, we found a significantly less pronounced reduction in media diameter in LF 08–299 prophylactically treated animals 4 months after transplan-

tation, especially in those with higher doses of the agent, compared with CyA-treated animals or untreated controls (Table 3).

Discussion and conclusions

The new potent immunosuppressive agent, LF 08–299, an analogue of 15-deoxyspergualin, was tested for its efficacy to prevent or treat chronic rejection in the aortic allograft model in rats. Despite the ability of LF 08–299 to induce permanent acceptance of fully mismatched rat cardiac allografts, we did not find a significant reduction in intimal proliferation, as a main marker of vasculopathy in chronically rejected organs, or a reduction in cell infiltration. We found a less pronounced reduction in media in LF 08–299 prophylactically treated animals using an optimal dose. In view of the capability of LF 08–299 to induce long-term fully mismatched heart graft acceptance, the low efficacy of this treatment is hard to explain. We concluded that LF 08–0299 is only able to reverse the reduction in media thickness in aortic allografts, but not intimal proliferation in this model of chronic rejection. Further studies in another experimental model of chronic rejection are necessary to answer finally the question about efficacy of LF 08–299 in chronic rejection of parenchymal organ transplantation.

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