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Hongsi Jiang
Shiro Takahara
Dan Li
Mikio Namiki
Michio Ishibashi
Akihiko Okuyama
Takao Sonoda

The superior effect of the combination of FK 506 and deoxyspergualin on rat cardiac allograft survival

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H. Jiang[1] (✉) · S. Takahara · D. Li
N. Namik · M. Ishibashi · A. Okuyama
T. Sonoda
Department of Urology, Osaka University Hospital, Yamadaoka 2-2, Suita-Shi, Osaka, Japan

[1] *Present address:* Division of Immunology, Product Development Laboratories, Fujisawa Pharmaceutical CO., Ltd.

Abstract In this present study, the effects of FK 506 and 15-deoxyspergualin (DSG), with respect to dose, timing, and combination, were investigated in an ACI-to-LEW rat cardiac allograft model. FK 506 was adminstered intramuscularly for 14 days starting on day 0 after grafting, while DSG was given intraperitoneally for 7 days starting on day 0, 4, or 7 after transplantation. FK 506 or DSG monotherapy prolonged cardiac allograft survival in dose-dependent manners, and the minimum effective dose for overcoming rejection was 0.1 mg/kg per day in the case of FK 506 and 1.0 mg/kg per day for DSG. The graft survival rate was higher with administration of DSG starting on day 4 on day 0 after transplantation. A low dosage of FK 506 strating on day 0, in combination with DSG starting on day 0 or day 4 (but not on day 7), had a synergistic effect in prolonging allograft survival for 14.0 ± 3.3 days and 25.4 ± 8.2 days, respectively. The most effective combination treatment schedule for prolongation of allograft survival was FK 506 starting on day 0 and DSG starting on day 4 after transplantation.

Key words FK 506, deoxyspergualin, heart transplantation · Rat, heart, immunosuppression
Deoxyspergualin, FK 506, rat heart
Heart transplantation, rat, FK 506, deoxyspergualin

Introduction

FK 506 was first isolated from *Streptomyces tsukubaensis* as an antibiotic [3, 9] and 15-deoxyspergualin (DSG) is a derivative of spergualin, which was isolated from *Bacillus laterosporus* as an anticancer agent [13]. Both have been shown to possess a potent immunosuppressive effect in vitro and in experimental organ transplantation. It has also been reported that these drugs are useful for immunosuppression in clinical organ transplantation and in patients with autoimmune diseases [4, 8].

However, various side effects of FK 506, including nephrotoxicity, neurotoxicity, and diabetogenicity, have been noted in clinical trials [2], and DSG cannot be administered in an oral form and used as primary therapy for organ transplantation [1, 8]. One approach to solving these problems is to optimize drug therapy by utilizing the minimum effective dose, choosing the appropriate timing of administration, or administering combination therapy [10]. In the present study, we investigated the above-mentioned factors using FK 506 and DSG in a rat cardiac allograft model.

Materials and methods

Animals

Male Lewis (LEW) rats (RT1^l) weighing 250–300 g were used as the recipients. Male LEW and male ACI rats (RT1^a) weighing 150–200 g were used as the syngeneic and allogeneic donors, respectively. The animals were obtained from commercial sources (LEW rats from Charles River, Japan, and ACI rats from Hoshino Experiment Animals, Japan) and kept under specific, pathogen-free conditions in our animal facility.

Table 1 ACI rat cardiac graft survival in LEW recipient rats treated with FK 506 and DSG

Group		Drug	Period (post-transplantation)	*n*	Survival days (*n*)[a]	Mean ± SD	*P* value
Syngeneic graft		(–)	(–)	6	> 50	> 50	
Allogeneic graft		(–)	(–)	6	5, 6 (3), 7 (2)	6.2 ± 0.8	
1	FK 506	0.32 mg/kg	Days 0–13	5	10, 15 (2), 18, 24	16.4 ± 5.1	< 0.001[b]
2		0.1	0–13	5	8, 10, 12 (2), 15	11.4 ± 2.6	< 0.01
3		0.032	0–13	5	6, 7 (2), 9, 11	8.0 ± 1.3	NS
4	DSG	2.5 mg/kg	Days 0–6	5	8, 10 (2), 11, 13	10.4 ± 1.8	< 0.001 / < 0.05[c]
5		2.5	4–10	5	12 (2), 14, 15, 17	14.0 ± 2.1	< 0.001
6		1.0	0–6	5	7, 9, 10, 11 (2)	9.6 ± 1.6	< 0.01 / NS
7		1.0	4–10	5	9, 10, 11 (2), 13	10.8 ± 1.4	< 0.001

[a] Numbers in parentheses are numbers of individuals surviving to that day
[b] Mean comparison between allograft control group and immunosuppressant-treated group
[c] Mean comparison between administration of DSG starting on day 0 and day 4 after transplantation

Heterotopic heart transplantation

Heart transplantation was performed using the modified technique of Ono and Lindsey [12], with the donor aorta and pulmonary artery anastomosed end-to-side to the recipient's abdominal aorta and inferior vena cava, respectively. Cardiac allograft survival was determined by daily palpation. Rejection was considered complete at the time of cessation of a palpable heartbeat and was confirmed by histological examination.

Drug preparation

FK 506 was supplied by Fujisawa (Osaka, Japan) and DSG by Nihon-Kayaku (Tokyo, Japan). Both drugs were dissolved in physiological saline before use. FK 506 was administered intramuscularly and DSG was given intraperitoneally.

Experimental groups

Rats subjected to heart transplantation were divided into ten groups, as follows:

Syngeneic control ($n = 6$)

Allogeneic control ($n = 6$)

Group 1 ($n = 5$) FK 506, 0.32 mg/kg per day, from day 0 to day 13

Group 2 ($n = 5$) FK 506, 0.1 mg/kg per day, from day 0 to day 13

Group 3 ($n = 5$) FK 506, 0.032 mg/kg per day, from day 0 to day 13

Group 4 ($n = 5$) DSG, 2.5 mg/kg per day, from day 0 to day 6

Group 5 ($n = 5$) DSG, 2.5 mg/kg per day, from day 4 to day 10

Group 6 ($n = 5$) DSG, 1.0 mg/kg per day, from day 0 to day 6

Group 7 ($n = 5$) DSG, 1.0 mg/kg per day, from day 4 to day 10

Group 8 ($n = 5$) FK 506, 0.032 mg/kg per day, from day 0 to day 13 + DSG, 1.0 mg/kg per day, from day 0 to day 6

Group 9 ($n = 5$) FK 506, 0.032 mg/kg per day, from day 0 to day 13 + DSG, 1.0 mg/kg per day, from day 4 to day 10

Group 10 ($n = 5$) FK 506, 0.032 mg/kg per day, from day 0 to day 13 + DSG, 1.0 mg/kg per day, from day 7 to day 13

Statistical analysis

Differences in graft survival between the various groups were analyzed using Student's *t*-test. A *P* value below 0.05 was considered significant difference.

Results

Control groups

Syngeneic grafts survival for more than 50 days, while the average graft survival time in the allogeneic control group was 6.2 ±0.8 days (Table 1).

FK 506 and DSG monotherapy groups

FK 506 monotherapy prolonged cardiac allograft survival in a dose-dependent manner with the lowest effective dosage being 0.1 mg/kg per day. DSG also prolonged cardiac allograft survival with a dose-dependent response when given on days 0–6 or days 4–10 after transplantation, although the graft survival rate was higher with DSG on days 4–10. The lowest effective dose for such an effect was 1.0 mg/kg per day (Table 1).

Table 2 ACI rat cardiac graft survival in LEW recipient rats treated with low-dose FK 506 in combination with DSG

Group	Drug		Period (post-transplantation)	*n*	Survival days (*n*)[a]	Mean ± SD	*P* value
3	FK 506	0.032 mg/kg	Days 0–13	5	6, 7 (2), 9, 11	8.0 ± 1.3	
6	DSG	1.0 mg/kg	0–6	5	7, 9, 10, 11 (2)	9.6 ± 1.6	
7		1.0 mg/kg	4–10	5	9, 10, 11 (2), 13	10.8 ± 1.4	
8	FK 506 DSG	0.032 mg/kg 1.0 mg/kg	0–13 0–6	5	11, 14 (2), 15, 20	14.8 ± 3.3	< 0.01[b] < 0.05[c] < 0.05[d]
9	FK 506 DSG	0.032 mg/kg 1.0 mg/kg	0–13 4–10	5	13, 24, 26, 28, 36	25.4 ± 8.2	< 0.01[b] < 0.01[c] < 0.01[d]
10	FK 506 DSG	0.032 mg/kg 1.0 mg/kg	0–13 7–13	5	7, 8, 13, 18, 19	13.0 ± 5.5	NS

[a] Numbers in parentheses are numbers of individuals surviving to those days
[b] Mean comparison between group 3 and the combination-treated group
[c] Mean comparison between group 6 and the combination-treated group
[d] Mean comparison between group 7 and the combination-treated group

FK 506 + DSG-rated groups

FK 506 (0.032 mg/kg per day for 14 days) in combination with DSG (1.0 mg/kg per day for 7 days) demonstrated a synergistic effect on cardiac allograft survival in groups 8 and 9, but not in group 10 (Table 2). The duration of graft survival was 14.0 ± 3.3 days with administration of FK 506 on days 0–13 and DSG on days 0–7 post-transplantation, and it was 25.4 ± 8.2 days with FK 506 given on days 0–13 and DSG on days 4–10. However, graft survival decreased to 13.0 ± 5.5 days with the administration of FK 506 on days 0–13 and DSG on days 7–13 after grafting. The most effective combination treatment schedule for prolonging allograft survival was the administration of FK 506 starting on day 0 and DSG starting on day 4 after transplantation.

Discussion

The present study confirmed that both FK 506 and DSG dose-dependently prolonged cardiac allograft survival in the rat. The lowest dose for such an effect was 0.1 mg/kg per day in the case of FK 506 and 1 mg/kg per day for DSG. These results agree with those reported previously [7, 11].

In addition, a dramatic in vivo synergy between low-dose FK 506 and DSG, which resulted in the prolongation of allograft survival, was displayed in the present study. While the mechanism of the synergistic interaction between these two drugs is still uncertain, it might be based on their different structures and modes of immunosuppression. Structurally, FK 506 is a macrolide lactone, while DSG is a straight polypeptide. Functionally, FK 506 inhibits the early phase of the lymphocyte response, especially lymphokine synthesis, while DSG only suppresses the late phase of the mixed lymphocyte reaction (MLR) by inhibiting IL2-receptor expression. In addition, FK 506 suppresses the mitogen-stimulated lymphocyte blastogenesis, while DSG has no such effect, and FK 506 suppresses the activation (but not the differentiation) of B lymphocytes, while DSG selectively suppresses the early differentiation process of B lymphocytes [5, 8, 12]. Thus, FK 506 and DSG are likely to influence the in vivo process of cardiac allograft rejection in different ways and may, therefore, induce a synergistic effect on allograft survival. Our previous study on the in vitro immunosuppressive effect of combination therapy also strongly supports the present experimental results. In our earlier study, DSG showed almost no suppression as a single agent but displayed on additive suppressive effect on the PHA and CD3-induced lymphocyte responses when combined with FK 506 [6]. These findings strongly suggest that the effect of combination therapy in vivo is closely related to the in vitro effect. Synergistic effects may occur through both chemical and biological interactions between two drugs. Moreover, no side effects were noted in the recipient rats treated with this combination therapy, although anorexia and emaciation were encountered with high-dose FK 506 treatment in the same experimental system (data not shown).

An interesting finding in this study was that, in the case of monotherapy, cardiac allograft survival was prolonged better by administration of DSG on days 4–10 than on days 0–6 after transplantation. In addition, the most effective schedule for combination therapy was the administration of FK 506 on days 0–13 and DSG on days 4–10 after transplantation, which induced allograft survival of 25.4 ±8.2 days, although a synergistic effect was also demonstrated by administration of FK 506 on

days 0–13 and DSG on days 0–6. Allograft rejection usually starts on day 4 after grafting, and it is generally difficult to control rejection-associated lymphocytes using conventional immunosuppressive agents. These in vivo results run parallel to those of our previous human in vitro study of deoxymethylspergualin (MeDSG), a stable analogue of DSG. In that study, MeDSG suppressed the human MLR response even when added only in the final 24 h before ^{3}H-TdR incorporation, and the degree of suppression was not significantly different from that when the drug was added on day 0 at the same doses [5]. Our finding suggested that DSG could selectively suppress activated T lymphocytes during the acute phase of allograft rejection, and that the cardiac allograft therefore showed synergistic prolongation of survival when administration of DSG started during the ongoing acute rejection period rather than immediately after transplantation or in the late phase of rejection.

In conclusion, we confirmed that FK 506 or DSG monotherapy could induce prolongation of rat cardiac allograft survival in a dose-dependent manner. Moreover, we are the first to show that a combination of low-dose FK 506 and DSG can further potentiate graft survival. It might be advantageous to use FK 506 as a primary prophylactic agent and DSG for antirejection treatment in clinical organ transplantation.

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