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Sensitization interval and administration method alter the effect of 15-deoxyspergualin on heart transplantation in sensitized recipient rats

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Abstract We evaluated the effect of 15-deoxyspergualin (DSG) on accelerated rejection. Brown Norway rats (BN) served as organ donors and Lewis rats (LEW) as recipients. In an accelerated rejection model, after a LEW rat was sensitized with BN skin, a BN heart was transplanted. Various intervals between sensitization and heart transplantation were examined. The heart allografts in sensitized recipients were rejected earlier than those in unmodified recipients regardless of the sensitization interval. DSG (2.5 mg/kg per day), given to the recipients during the sensitization phase, significantly prolonged graft survival compared with the untreated hosts when the sensitization interval was short. When the recipients were treated with DSG after heart transplantation, heart graft survival was significantly prolonged regardless of the sensitization interval. Flow cytometric analysis and complement-dependent cytotoxicity tests revealed that DSG suppressed antidonor antibody formation and that postoperative administration of DSG significantly decreased the proliferation of B cells when the sensitization interval was short and the proliferation of class II antigen-positive cells when the sensitization interval was long.

Key words 15-Deoxyspergualin, rat, heart transplantation · Heart transplantation, rat, 15-deoxyspergualin

Introduction

When a recipient is sensitized with donor antigens, a transplanted organ tends to be rejected earlier than in acute rejection [11, 16]. In clinical transplantation, this type of rejection is called hyperacute or accelerated rejection, depending on the rejection velocity [7]. These types of rejection occur in recipients with a history of presensitization, such as blood transfusion, pregnancy, or previous organ transplantation [21]. Pre-existing antibodies against donor antigens are responsible for these rejection reactions.

Spergualin is an antibiotic extracted from the culture filtrate of *Bacillus laterosporus* [27]. 15-Deoxyspergualin (DSG) is a derivative of spergualin and is reported to be a potent immunosuppressive agent with a beneficial effect on allograft survival in not only experimental but also clinical transplantation [1–3, 9, 22, 29, 30].

There are many reports about the mechanisms of immunosuppressive action of DSG, which include the suppression of macrophage function by decreasing MHC class II antigen expression [4]; suppression of T-cell function, including mixed lymphocyte reaction (MLR), plaque-forming cell (PFC) reaction against sheep red blood cells [3, 4] and cytotoxic T-cell activity [18]; and sparing of donor-specific suppressor cells [23]. There are few reports about the influence of this drug on organ transplantation in sensitized recipients [6, 28].

In this study, we examined the effect of the interval between sensitization of the recipient and transplantation on the rejection velocity. We also evaluated whether DSG effectively inhibited the accelerated rejec-

tion, and we analyzed the methods of administering the drug.

Materials and methods

Animals

Brown Norway rats (BN, $RT1^n$) were used as organ donors and Lewis rats (LEW, $RT1^l$) as recipients. All rats were males. LEW rats consistently received BN heart allografts 7 months after birth. Brown Norway rats were purchased from Shimizu Laboratory Supplies (Kyoto, Japan) and Lewis rats from Charles River (Kanagawa, Japan).

Skin grafting

Two BN skin flaps (3 cm × 3 cm each) were cleansed of subcutaneous tissue and grafted to the bilateral flank regions of the LEW recipient by interrupted suture using 2–0 silk (Nihonshoji, Osaka, Japan).

Heterotopic heart allotransplantation

The BN heart was removed after being perfused with 4 °C cold saline. The aorta and pulmonary artery of the donor heart were anastomosed to the abdominal aorta and the inferior vena cava of the recipient, respectively, using standard microvascular techniques.

Graft rejection was assessed by cessation of the ventricular beat by daily palpation.

Accelerated rejection model

After the LEW recipient was sensitized with BN skin, the BN heart graft was transplanted into the recipient either 2 weeks, 1 month, 3 months, 5 months, or 6 months later. Allograft survival in each group was compared.

Administration of DSG in the accelerated rejection model

15-Deoxyspergualin (DSG) was intraperitoneally given to the recipients at a dose of 2.5 mg/kg per day following either of two protocols: in group 1, 7 consecutive days prior to heart transplantation or in group 2, 7 consecutive days after heart transplantation. In the control group, the BN hearts were transplanted into the sensitized recipients without DSG treatment.

Spleen cell preparation and phenotypic analysis by flow cytometry

The spleen obtained from the recipient was diced and minced in Hanks balanced salt solution (HBSS, GIBCO, Grand Island, N.Y., USA) including 10 % Nu serum (Collaborative Research, Medford, Mass., USA). Then, 0.83 % ammonium chloride solution was added to splenic cells in order to remove red blood cells. After the suspension was washed, the splenic mononuclear cells were incubated with the following antibodies (mAbs) purchased from Serotic (Oxford, England): OX3 (class II antigen-positive cells), W3/25 (T helper/inducer cells), OX8 (T suppressor/cytotoxic cells), and OX33 (B cells). After 1 h of incubation, these cells were washed three times and then labeled with FITC-conjugated goat anti-mouse IgG antibody (noncross-reactive with rat IgG; Cappel, Westchester, Pa., USA) for 1 h at 4 °C. The percentage of antibody-positive cells was calculated with a fluorescence-activated cell sorter (CS-20; Showa Denko KK, Japan).

Table 1 Heart graft survival in sensitized recipients (*MST*, mean survival time)

Sensitization interval	Allograft survival (days)	MST ± SD	P^a
2 Weeks	4.4.4.4.5	4.2 ± 0.5	< 0.0001
1 Month	5.5.5.5.5	5.0 ± 0	< 0.0001
3 Months	5.5.5.5.5	5.0 ± 0	< 0.001
5 Months	5.5.5.6.6	5.4 ± 0.6	< 0.001
6 Months	5.5.5.5.6	5.2 ± 0.5	< 0.0001
Sensitization (–)	6.7.7.7.7.7	6.8 ± 0.4	

[a] Statistical significance between sensitized recipient and unmodified host

Complement-dependent cytotoxicity (CDC) test

Twofold, diluted recipient serum (50 µl) was added to BN lymph node cells (2.5×10^5) in 50 µl RPMI 1640 medium (GIBCO). After 1 h of incubation at 5 °C, rabbit complement (1 : 10, 50 µl) was added to each well. After 75 min of incubation at 37 °C, the percentage of injured lymphocytes was calculated by means of the dye exclusion method.

Histopathological evaluation of heart allografts

Rejected heart allografts were removed, fixed in 10 % formaldehyde, and stained with hematoxylin and eosin (HE).

Statistical analysis

Student's *t*-test was used for the statistical analysis. The difference was considered significant at a P value below 0.05.

Results

Allograft survival based on the sensitization interval (Table 1)

BN heart allografts were acutely rejected at an average of 6.8 ± 0.4 days in unmodified LEW recipients. LEW recipients sensitized with BN skin 2 weeks, 1 month, 3 months, 5 months, or 6 months before receiving BN heart allografts rejected the heart allografts consistently faster than nonsensitized hosts.

Effect of DSG on allograft survival in sensitized recipients (Table 2)

Administration of DSG for 7 consecutive days prior to heart transplantation (group 1)

DSG prolonged allograft survival significantly when the sensitization interval was relatively short (2 weeks, 1 month). In contrast, DSG did not influence allograft survival when the sensitization interval was longer than 3 months.

Administration of DSG for 7 consecutive days after heart transplantation (group 2)

DSG significantly extended allograft survival in all recipients, regardless of the sensitization interval, as compared with the sensitized recipients without DSG treatment.

Analysis of surface antigens on splenic mononuclear cells in sensitized recipients (Table 3)

Spleen cells were taken from recipients immediately before transplantation and 2 days after transplantation. The percentages of OX3 (+) cells (class II antigen-positive cells) and OX33 (+) cells (B cells), as well as the CD4/CD8 ratio, were evaluated.

Short sensitization interval (2 weeks)

In untreated recipients, the percentages of class II antigen-positive cells and B cells remained unchanged before and after heart transplantation. In group 1, the percentage of B cells decreased immediately before transplantation, but increased to a value comparable to that in the untreated group on the 5th postoperative day. In group 2, the percentage of B cells significantly decreased 5 days after surgery (26.3 % ± 0.7 % vs 8.9 % ± 2.4 %, $P < 0.05$).

The CD4/CD8 ratio remained unchanged even after the BN heart was transplanted into the sensitized recipient in the control group. However, DSG significantly decreased the CD4/CD8 ratio after the sensitized recipients received heart allografts when the drug was given either during the sensitization phase or after heart transplantation (3.31 ± 0.1 vs 2.83 ± 0.3 in group 1, 2.52 ± 0.4 vs 1.35 ± 0.2 in group 2).

Table 2 Effect of 15-deoxyspergualin (DSG) on heart graft survival (*MST*, mean survival time)

Sensitization interval	DSG	Graft survival (days)	MST ± SD	P^a
Group 1				
2 Weeks	(+)	7. 7. 7. 7. 8	7.2 ± 0.5	< 0.01
	(−)	4. 4. 4. 4. 5	4.2 ± 0.5	
1 Month	(+)	6. 6. 6. 7. 7	6.4 ± 0.6	< 0.01
	(−)	5. 5. 5. 5. 5	5.0 ± 0	
3 Months	(+)	5. 5. 5. 5. 5	5.0 ± 0	NS
	(−)	5. 5. 5. 5. 5	5.0 ± 0	
5 Months	(+)	5. 5. 5. 6. 6	5.4 ± 0.6	NS
	(−)	5. 5. 5. 6. 6	5.4 ± 0.6	
6 Months	(+)	6. 6. 6. 6. 6	6.0 ± 0	NS
	(−)	5. 5. 5. 5. 6	5.2 ± 0.5	
Group 2				
2 Weeks	(+)	8.11.11.11.12	10.6 ± 1.5	< 0.01
	(−)	4. 4. 4. 4. 5	4.2 ± 0.5	
1 Month	(+)	9. 9. 9.10.10.10	9.5 ± 0.6	< 0.01
	(−)	5. 5. 5. 5. 5	5.0 ± 0	
3 Months	(+)	9. 9.10.10.10.11	9.8 ± 0.8	< 0.01
	(−)	5. 5. 5. 6. 6	5.4 ± 0.6	
5 Months	(+)	10.10.10.11.11	10.4 ± 0.6	< 0.01
	(−)	5. 5. 5. 5. 6	5.2 ± 0.5	
6 Months	(+)	11.11.11.11.13	11.4 ± 0.9	< 0.01
	(−)	5. 5. 5. 5. 6	5.2 ± 0.5	

[a] Comparison between DSG-treated recipient and untreated host

Long sensitization interval (6 months)

In untreated recipients, the percentage of B cells significantly increased postoperatively.

In group 1, the percentages of class II antigen-positive cells and B cells significantly decreased immediately before heart transplantation, but then increased to values comparable to those in the untreated hosts.

In group 2, the percentage of class II antigen-positive cells significantly decreased on the 5th day after heart transplantation (23.3 % ± 0.8 % vs 18.2 % ± 2.4 %, $P < 0.05$).

The CD4/CD8 ratio remained unchanged regardless of DSG administration to the sensitized recipient.

Complement-dependent cytotoxicity (CDC) test (Fig. 1)

The CDC test revealed that preoperative or postoperative administration of DSG significantly suppressed the cytotoxicity level of recipient serum when the sensitization interval was short. Postoperative administration of DSG suppressed the cytotoxicity level of recipient serum more effectively than preoperative administration of DSG. In contrast, post-transplant administration of DSG consistently suppressed the cytotoxicity level of recipient serum when the sensitization interval was long.

Table 3 Analysis of surface antigens on splenic mononuclear cells in sensitized recipients (*Tx* heart transplantation)

Group	OX3 (+) cells (%)		OX33 (+) cells (%)		CD4/CD8	
	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx
Sensitization interval: 2 weeks						
Untreated	30.8 ± 4.4	29.5 ± 3.1	26.3 ± 0.7	27.5 ± 2.1	2.52 ± 0.4	2.25 ± 0.1
1	30.9 ± 3.1	31.8 ± 3.5	23.2 ± 1.0	27.8 ± 1.9	3.31 ± 0.1	2.83 ± 0.3
			*		*	
2	30.8 ± 4.4	23.2 ± 4.3	26.3 ± 0.7	8.9 ± 2.4	2.52 ± 0.4	1.35 ± 0.2
			*		**	
Sensitization interval: 6 months						
Untreated	23.0 ± 0.8	24.2 ± 1.6	24.7 ± 1.8	31.7 ± 0.9	3.49 ± 0.3	3.97 ± 0.4
			*			
1	17.5 ± 0.9	22.9 ± 1.6	15.3 ± 2.0	21.3 ± 0.8	3.70 ± 0.8	3.95 ± 0.7
	*		*			
2	23.3 ± 0.8	18.2 ± 2.4	24.7 ± 1.8	21.9 ± 1.5	3.49 ± 0.3	4.01 ± 0.5
	*					

* $P < 0.05$; ** $P < 0.001$

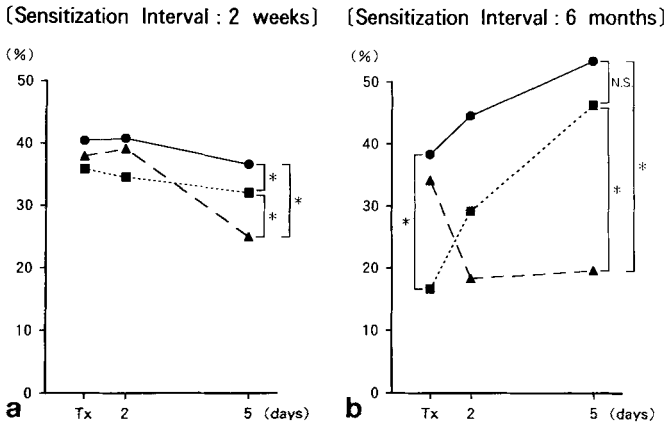

Fig. 1. a, b Complement-dependent cytotoxicity test in sensitized recipients. Serum was obtained from the sensitized recipient with or without DSG treatment. A mean of four to five measurements at each time point is shown. * $P < 0.05$ (● control group, ■ group 1, ▲ group 2)

Histopathological evaluation of heart allografts

Histological examination of rejected heart allografts in sensitized recipients who received no DSG revealed mainly hemorrhage and necrosis of the myocardium associated with neutrophil infiltration, irrespective of the sensitization interval. In contrast, sensitized recipients treated with DSG postoperatively showed no appreciable evidence of myocardial hemorrhage or necrosis.

Discussion

It has been reported that sensitized recipients have a higher rate of graft rejection than unsensitized recipients [10, 26]. In this experiment, it was found that heart allografts in sensitized recipients were rejected earlier than those in unsensitized recipients regardless of the interval between sensitization and heart transplantation. Clinical transplantation requires the exercise of care in the management of organ transplant recipients with a history of sensitization, regardless of the amount of time that has passed since the sensitization. Graft rejection in sensitized recipients is known to be mediated by pre-existing antidonor cytotoxic antibodies, and grafted tissue is damaged by fixation of complement to the antibody [19].

In managing organ transplantation in sensitized recipients, there are two ways of administering immunosuppressive agents: during the sensitization period, to modify the sensitization status of the recipient, and after transplantation.

There are many reports about methods of immunosuppression and immunomodulation of graft rejection in sensitized recipients. Selective lymphoid irradiation combined with antilymphocyte globulin (ALG) was found to delay cardiac allograft rejection with suppression of the CDC level [8]. Cyclosporin A was effective in prolonging cardiac allograft survival in sensitized mice when given after sensitization but before engraftment [13]. Some monoclonal antibodies have also successfully prolonged allograft survival in sensitized recipients, among them: anti-CD8 mAb (YTS 169.4) [14], anti-CD4 mAb (BWH-4) [20], and anti IL-2 receptor mAb (ART-18) [5]. However, these monoclonal antibodies were ineffective when used during the effector phase (after heart transplantation) in rats. When DSG was given to sensitized recipients, its effects on graft survival differed according to the length of the sensitization interval.

In this experiment, preoperative administration of DSG extended allograft survival when the sensitization interval was short. In contrast, postoperative administration of DSG prolonged graft survival significantly, regardless of the sensitization interval.

Flow cytometric analysis demonstrated that DSG treatment during the sensitization period significantly suppressed B cell proliferation, resulting in the inhibition of antidonor antibody formation when the sensitization interval was short. In contrast, DSG given to the recipient during the sensitization period had no effect on graft survival when the sensitization interval was long. That may be because memory B cells already existed in the case of long sensitization intervals. It was therefore suggested that DSG (2.5 mg/kg per day × 7) was not sufficient to prevent the formation of antibodies released from these cells. Memory B cells may be less sensitive to DSG than B cells during the primary immunoresponse. Tepper et al. examined the effect of DSG on humoral immunity using highly immunogenic antigens [24]. They reported that while DSG was highly effective in inhibiting the primary immunoresponse and the development of memory, it was less effective in inhibiting an established immunoresponse. In other experimental studies, DSG has been shown to inhibit antibody responses [15, 17, 25]. When DSG was given to recipients after heart transplantation, it successfully suppressed the proliferation of B cells (sensitization interval 2 weeks) and class II antigen-positive cells (sensitization interval 6 months), resulting in prolonged allograft survival.

The CD4/CD8 ratio decreased in rats that received DSG either during the sensitization phase or after heart transplantation when the sensitization interval was relatively short. In group 1, the decrease in the CD4/CD8 ratio resulted from the decrease in the percentage of CD4-positive cells. In contrast, the increase in the percentage of CD8-positive cells was responsible for the decrease in the CD4/CD8 ratio in group 2. However, the CD4/CD8 ratio remained unchanged, and such a phenomenon was not observed when the sensitization interval was long. DSG has been reported to induce or spare donor-specific suppression of T cells in allotransplantation [12]. In this experiment, suppressor cells may have played a role in graft prolongation in the sensitized recipient when the drug was administered after the operation.

In conclusion, DSG consistently prevented accelerated rejection, regardless of the sensitization interval, when it was given to sensitized recipients postoperatively.

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