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Therapeutic effect of 15-deoxyspergualin on acute graft rejection in canine liver transplantation

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Abstract Therapeutic effect of 15-deoxyspergualin (DSG) on acute rejection was investigated by examining hepatic functions and histological findings in a model of canine liver transplantation. When acute rejection (defined as an acute rise of hepatic functions) occurred, the recipients were treated with DSG alone or combined with a small amount of methylprednisolone (MP). The recipients in group 1 were administered no basic immunosuppressant, and those in groups 2 and 3 received cyclosporine as the basic drug. The rejections in groups 1 and 2 were treated with DSG combined with MP and those in group 3, with DSG alone. Amelioration or healing of hepatic dysfunction and histological abnormalities as seen in all groups except for one case in group 3. The observations in this study were quite similar to those in renal transplantation. Therefore, DSG therapy is expected to be useful for treating graft rejection even in clinical liver transplantation.

Key words 15-Deoxyspergualin
Pulse therapy · Liver transplantation
Canine model

Introduction

15-Deoxyspergulain (DSG) is a new immunosuppressant isolated from culture filtrate of *Bacillus laterosporus* [1]. The immunological mechanisms of action of DSG have been reported as specific inhibition of expanded lymphocyte clones in an acute graft rejection, preservation of suppressor cells [2], and inhibition of cytotoxic T lymphocytes and of the differentiation and proliferation of B lymphocytes [3]. Therefore, DSG has been proposed for use in the treatment of acute graft rejection, and indeed, we have observed the efficacy of the drug in experimental models of heart and renal transplantations in rats and dogs [2, 4], as well as in clinical trials of renal transplantation [5]. In this study, we investigated the efficacy of DSG alone or DSG in combination with a small amount of methylprednisolone (MP) for ongoing rejection in canine liver grafting.

Materials and methods

Animals

Adult mongrel dogs weighing 10–12 kg were used as donors and adult beagles with a similar weight as recipients. The animals were administered 0.003 mg sulfate atropine kg and anesthetized with 25 mg sodium pentobarbital kg; their breathing was maintained with a respirator.

Liver transplantation

After an intravenous injection of heparin (2000 U) in the donor animals, liver perfusion was performed via the portal vein with

lactate Ringer's solution (4 °C, 1000 ml). The liver was then removed with the abdominal aorta and stored in saline solution at 4 °C. Two passive venovenous bypasses were placed in recipients and their liver was removed. The liver grafting was conducted as follows. The suprahepatic vena cava was anastomosed first, thereafter the portal vein was reconstructed, and then the portal blood flow was released. Reconstruction of the infrahepatic vena cava was performed, and recirculation of the common hepatic artery was obtained by anastomosis of the donor and recipient aorta in an end-to-side manner. Finally, the biliary reconstruction was done by anastomosis of the gal bladder to the duodenum.

Experimental protocol

Hepatic function, which was assessed by aspartate aminotransferase (AST) and total bilirubin (TB) levels in the recipient sera, was examined daily after grafting. When acute rejection, defined as an acute rise of AST and TB, occurred, the recipients were treated with DSG alone or combined with a small amount of MP. DSG and MP were intravenously injected into the recipients for 7 and 3 days, respectively, as a pulse therapy. The dosage of DSG was 4.8 mg/kg daily for the first 2 days, 2.4 mg/kg daily for the next 2 days, and 1.2 mg/kg daily for the last 3 days, and that of MP was 2.5 mg/kg daily. Cyclosporine (CsA) 5 mg/kg daily was intravenously injected into the recipients as a basic immunosuppressant every day from the day of transplantation. Liver biopsy was performed for histological examination before and after DSG therapy. The grade of graft rejection was classified by Demetri's criteria [6].

Experimental groups

Control group ($n = 3$): The recipients were treated with neither basic immunosuppressant nor pulse drug.

Group 1 ($n = 3$): The recipients were administered no basic immunosuppressant, but their acute rejection was treated with DSG combined with MP (DSG + MP).

Group 2 ($n = 5$): The recipients were administered CsA as a basic immunosuppressant and their acute rejection was treated with DSG + MP.

Group 3 ($n = 5$): The recipients were administered CsA, and their acute rejection was treated with DSG alone.

Statistics

For the analysis of the significance between the data, Student's t-test was used. P values less than 0.005 were considered significant.

Results

Results of transplantation and DSG therapy

As shown in Table 1, all graft rejections in the control group and group 1 occurred 4–5 days after grafting. Several graft rejections in groups 2 and 3 occurred 5–6 days after grafting, and others occurred 12–18 days after grafting. Recipient survival after DSG therapy in all groups was not significantly prolonged compared with the control group, but there was a tendency to prolonged survival. It was remarkable that rejection occurred more than 10 days after grafting.

Table 1 Results of transplantation and DSG therapy

Group	Survival (day)	Onset day of graft rejection	Survival after DSG therapy	
			(day)	(mean ± SD)
Control	8	5	3	2.67 ± 0.58
($n = 3$)	7	4	3	
	7	5	2	
Group 1	12	5	7	6.67 ± 1.53
($n = 3$)	12	4	8	
	9	4	5	
Group 2	12	5	7	15.8 ± 10.2
($n = 5$)	13	6	7	
	30	14	16	
	33	16	17	
	44	12	32	
Group 3	9	5	4	10.0 ± 5.24
($n = 5$)	12	5	7	
	14	6	8	
	28	13	15	
	34	18	16	

Changes in hepatic functions

As shown in Fig. 1, hepatic dysfunction in all animals of group 1 had been ameliorated from 2–3 days after DSG therapy, but they died before complete recovery. In group 2 dysfunction was more rapidly ameliorated from 2–3 days after DSG therapy. However, two animals in which rejection occurred within 1 week after grafting died before complete recovery, and three animals in which rejection occurred more than 10 days after grafting recovered completely, although they died later due to a recurrence of rejection or weakness. One case of rejection in group 3 died not recovered before death, while others slowly improved from 2–3 days after DSG therapy: two animals in which rejection occurred within 1 week after grafting died before complete recovery, and others in which rejection occurred, more than 10 days after grafting were almost completely recovered, although they died due to weakness.

Figure 2 shows the percentage changes of AST and TB on each day compared with those on the DSG therapy started in the improved cases of groups 2 and 3. Hepatic function in group 2 (rejection treated with DSG + MP) recovered more significantly and rapidly than that in group 3 (treated with DSG alone).

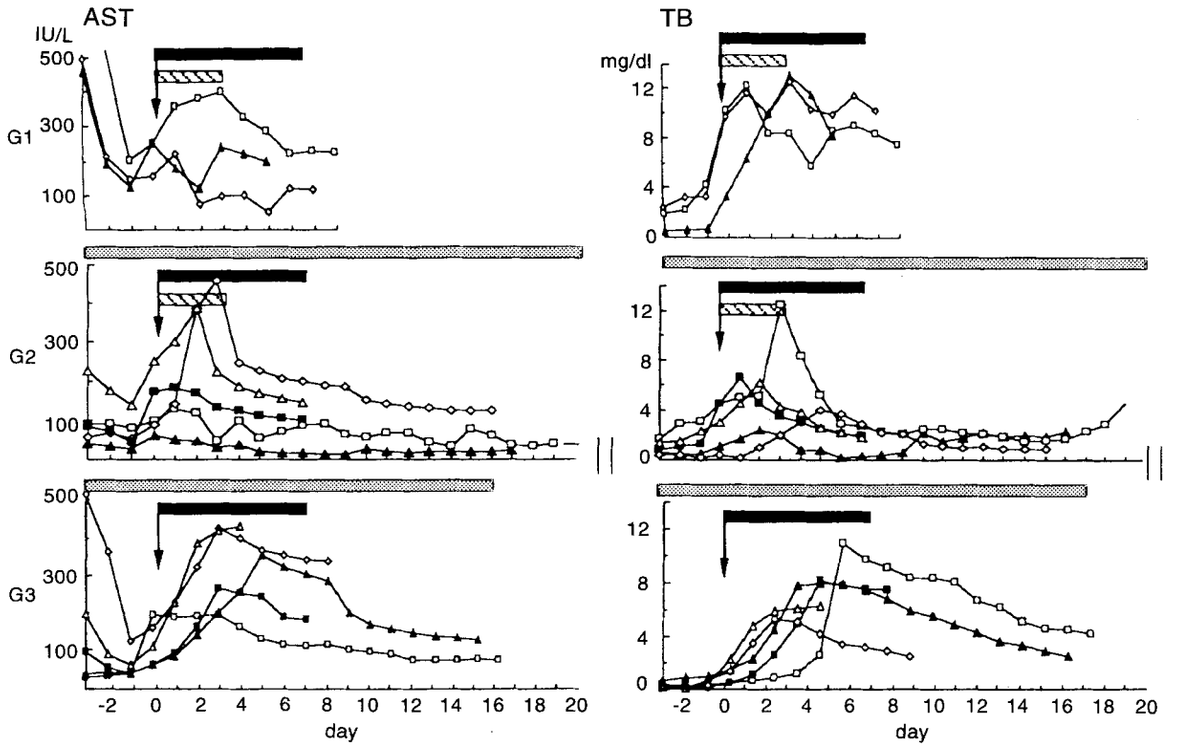

Fig. 1 Changes in hepatic functions after 15-deoxyspergulain (DSG) therapy (←) start of DSG therapy, ■ DSG, ▨ methylprednisolone, ▩ cyclosporin A, *G1* group 1, *G2* group 2, *G3* group 3)

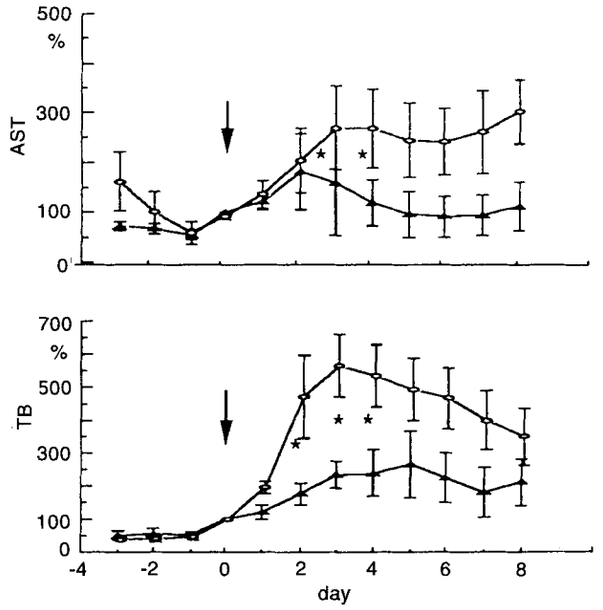

Fig. 2 Percentage changes in hepatic functions after DSG therapy (←) start of DSG therapy, —▲— 5 animals in group 2 treated with DSG + MP, —○— 4 animals in group 3 treated with DSG alone, * $P < 0.05$, *AST* aminotransferases, *TB* total bilirubin)

Histological examination

The histological grades of graft rejection before DSG therapy in groups 2 and 3, in which recipients were treated with CsA as a basic immunosuppressant, were milder than those in the control group and group 1 (not treated with basic immunosuppressant). According to the assessment of therapeutic effects based on the histological changes in graft rejection before and after DSG therapy, graft rejection was improved or healed in eight of nine animals; in four animals, the histological findings after DSG therapy were unknown.

Therapeutic effect and of day onset of graft rejection

Table 2 compares the therapeutic effect between eight animals in which rejection occurred within 1 week after grafting and five animals in which rejection occurred more than 10 days after grafting. Recipient survival in the latter was significantly longer after DSG therapy than that in the former. In addition, amelioration of the histological abnormalities in the latter was better than in the former in proportion to the difference of histological grade of graft rejection between both groups.

Table 2 Therapeutic effect and day of onset of graft rejection

Onset day of graft rejection	Survival after DSG therapy (mean ± SD; day)	Grade of graft rejection	Effect after DSG therapy
Within 7 days ($n=8$)	6.4 ± 1.4 [a]	Severe 1 Moderate 6 Mild 1	Improvement 3 Unchanged 1 Unknown 4
After day 10 ($n=5$)	19.2 ± 7.2 [b]	Moderate 2 Mild 3	Healing 1 Improvement 4

[a] vs [b] $P < 0.05$

Discussion

Treatment with steroids or a monoclonal antibody such as OKT-3 is commonly used in acute graft rejection, and the recovery rate from rejection is improving [7]. However, the recipients often suffered from severe side-effects from these drugs. Furthermore, rejections were sometimes resistant to steroid pulse therapy. A new rescue drug has been awaited. DSG, with its different immunosuppressive mechanism from the above-mentioned drugs [2, 3], has been examined for use in the treatment of graft rejection, and its efficacy has been demonstrated in experimental studies [2, 4] and clinical trials [5]. As for liver transplantation, experimental studies using it in a rat model [8, 9] and one clinical case study [10] have been reported, but the drug has not yet been fully examined, especially in large animals. Therefore, the present study was undertaken to investigate the efficacy of DSG therapy on canine liver grafting before clinical application to liver transplantation.

In his study, rejection therapy was always required in the recipients treated with CsA as the basic immunosuppressant. Acute graft rejection occurring in clinical recipients treated with CsA may be due to an insufficient blood level of the drug. It was therefore usual to raise the dosage of CsA to increase its blood level in order to aid the recovery of a mild graft rejection. In the present study, by contrast, CsA was not increased even after the onset of graft rejection, and consequently, it was fully expected that the graft rejection would progress or at least would not be ameliorated without further treatment.

The therapeutic effect of DSG was seen to vary among the recipients regarding the day of onset of graft rejection. The early occurrence of graft rejection indicated its severity, possibly due to a large difference in major histocompatibility complex antigens between the donor and recipient. In fact, the histological grades of graft rejection in those animals in which rejection occurred more than 10 days after grafting were milder than those in animals in which rejection occurred within 1 week after grafting, and the therapeutic effect in the former was significantly better than in the latter. It was suggested that DSG pulse therapy was effective in cases of relatively mild rejection. A similar result has been obtained in a study using canine renal trnsplantation [11]. In addition, early DSG therapy may seriously affect a recipient which had not completely recovered from the operative invasion. Acute graft rejection in clinical patients always occurred under conditions of sufficient immunosuppression and usually more than 2–3 weeks after grafting. Therefore, the cases in which rejection occurred more than 10 days after graftig seemed to be realistic models for clinical transplantation. From the favorable results in those cases, we anticipate that DSG therapy could be applied in clinical liver grafting.

The recovery of hepatic function was smoother in group 2 than in group 3. DSG is an antibiotic that does not have any antiinflammatory effect, and therefore DSG combined with a small amount of MP resulted in a remarkable recovery from acute rejection. These results were quite similar to those observed in recipients whose graft rejection was treated with DSG alone or combined with MP in clinical [12] and experimental [13] renal transplantations.

A direct toxic effect of DSG on the liver graft was not found in this study, judging from hepatic functions and histological findings. No severe side-effects have been reported except for mild gastrointestinal trouble and leukocytopenia in a clinical study of renal transplantation [5]. Furthermore, DSG is excreted by the kidney [14]. Thus, it seems therapy is DSG expecially beneficial in liver grafting.

In conclusion, DSG therapy was effective in the treatment of acute graft rejection in a model of canine liver transplantation. The observations in this study were quite similar to those in renal transplantation. Therefore, DSG therapy may be tried even in clinical liver transplantation.

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