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Effect of 15-Deoxyspergualin on Experimental Organ Transplantation

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15-DEOXYSPERGUALIN (DSPG) is a new antitumor and immunosuppressive agent derived from the antibiotic spergualin. This agent was shown to prolong the graft survival of skin, heart, pancreas islet, kidney, and liver in the rodent.[1-3] No observations have been made with large animals. In the present study, the effect of DSPG was first tested with rat heterotopic heart transplantation (HTX) and then evaluated with canine kidney transplantation (KTX).

MATERIALS AND METHODS

Animals

Lewis rats (RT 1) purchased from the Harlan Spraque Dawley Company (Indianapolis) were used as recipients. ACI rats (RT 1^a) were purchased from the Simonsen Lab (Gilcoy, CA) and used as donors.

Outbred beagle dogs were obtained from the University of Pittsburgh farm and were used as both donors and recipients.

Operative Procedures

HTX was performed by the method of Ono and Lindsay.[4] The donor aorta and pulmonary artery were anastomosed to the recipient aorta and infrarenal inferior vena cava with standard microvascular techniques. KTX was performed by conventional intra-abdominal techniques, with which the renal artery was anastomosed to the recipient iliac artery end-to-end and the renal vein was anastomosed to the recipient iliac vein end-to-side. Bilateral native nephrectomy was performed after graft ureteroneocystostomy.

Drug Administration

DSPG was supplied as a powder from the Nippon Kayaku Company, Ltd, Tokyo. It was dissolved in saline, and was given intraperitoneally to the rat for 2 weeks and intravenously to the dog for 50 days starting immediately after the transplant operation. In experiments with drug combinations in dogs, cyclosporine (CyA) was given orally in the commercial oil carrier that is used clinically. Oral prednisone (Pred) was given to dogs as a 5-mg tablet.

Experimental Groups

Intraperitoneal doses tested in HTX were 0.0 mg/kg/d (control), 0.5 mg/kg/d, 1.0 mg/kg/d, 3.0 mg/kg/d, and 6.0 mg/kg/d. Treatment was for 14 days beginning just after the operation. Rejection was diagnosed by the cessation of heartbeat and was confirmed visually by laparotomy and histologic examination.

The effect of intravenous DSPG alone and in combination with oral CyA and Pred on KTX were examined in the following groups:

1. Dose-effectiveness study
   - Group 1: (N = 6) DSPG (0.0 mg/kg/d)
   - Group 2: (N = 6) DSPG (0.25 mg/kg/d)
   - Group 3: (N = 6) DSPG (0.5 mg/kg/d)
   - Group 4: (N = 6) DSPG (0.75 mg/kg/d)
   - Group 5: (N = 6) DSPG (1.0 mg/kg/d)
2. Combination study
   - Group 6: (N = 10) DSPG (0.5 mg/kg/d) + CyA (5 mg/kg/d) + Pred (5 mg/d)
   - Group 7: (N = 6) DSPG (0.5 mg/kg/d) + CyA (5 mg/kg/d)
   - Group 8: (N = 6) DSPG (0.5 mg/kg/d) + Pred (5 mg/d)
   - Group 9: (N = 6) CyA (5 mg/kg/d) + Pred (5 mg/d)
   - Group 10: (N = 6) CyA (5 mg/kg/d)
   - Group 11: (N = 6) Pred (5 mg/d)

Because of emaciation of animals, the dose of DSPG in the combination experiments was reduced to 0.3 mg/kg/d after 3 weeks and to 0.2 mg/kg/d after 6 weeks. Blood samples were collected every three mornings for the determination of kidney and liver functions. Complete postmortem examination of animals was performed. His-

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Table 1. Survival of Grafts by Intraperitoneal Injection of DSPG

Group	Dose (mg/kg/d)	No.	Animals Died of Rejection (Day)	Median (Day)	*P* (<)	Animals Died of Infection
1	0.0	6	6, 6, 6, 7, 7	6	—	0/6
2	0.5	5	19, 20, 25, 29, 46	25	0.01	0/5
3	1.0	6	22, 27, 27, 35, 40	27	0.01	1/6
4	3.0	6	30, 32	—	—	4/6
5	6.0	6	—	—	—	6/6

NOTE. DSPG was given from day 0 to day 13 (14 days total).

tologic severity of graft rejection was determined blindly basing on subjective scales from 0 to + + +.

Statistics

Wilcoxon rank sum test and Student's *t* test were applied for the statistical analysis.

RESULTS

HTX

The median graft survival of untreated rats was six days (Table 1). Intraperitoneal DSPG administration at doses of 0.5 mg/kg/d and 1.0 mg/kg/d provided significant prolongation of graft survival. Higher doses were lethal. Four of six rats in group 4 and all animals in group 5 died of bronchopneumonia even though the grafts were beating. Colony contamination with pathogens could not be shown by serologic and microbiologic study of nontransplanted rats from the same laboratory environment. Thus, the deaths were attributed to over immunosuppression.

KTX

Dose-effectiveness study. Mean survival was 13.3 ± 3.6 (SD) days in untreated control dogs, (Fig 1) while that of treated dogs (groups 2 to 5) was variable. Significant prolongation of graft survival was obtained only with a dose of 0.75 mg/kg/d, group 4 (Fig 1), even though higher doses prevented creatinine (Cr) elevation (Fig 2) and even though histologic evidence of rejection was reduced at higher doses. The last Cr prior to animal death was 15.0 ± 4.6 (SD) mg/dL in group 1, 11.3 ± 3.8 in group 2, 5.4 ± 1.5 in group 3, 2.7 ± 2.8 in group 4, and 5.1 ± 3.4 in group 5. The prevention of Cr rise was significant in animals treated with 0.5 mg/kg/d or more of DSPG compared to groups 1 and 2. All animals given 1.0 mg/kg/d of DSPG died of lethal emaciation accompanied with severe diarrhea. Liver dysfunction was not observed in any group. Pulmonary congestion was the only pathologic abnormality in the extrarenal organs seemingly related to animal death.

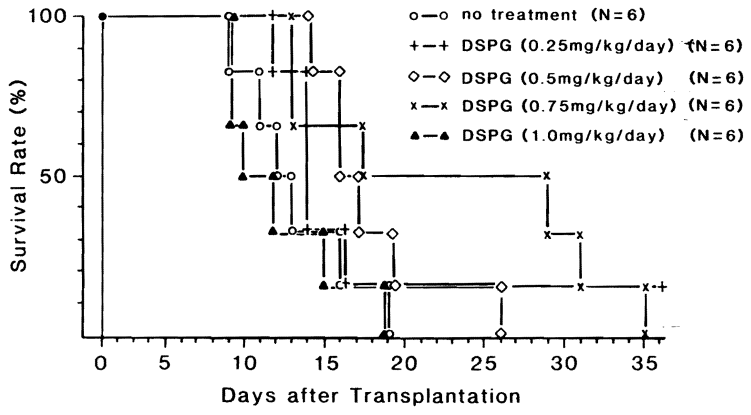

Fig 1. Survival of KTX dogs treated with different doses of DSPG.

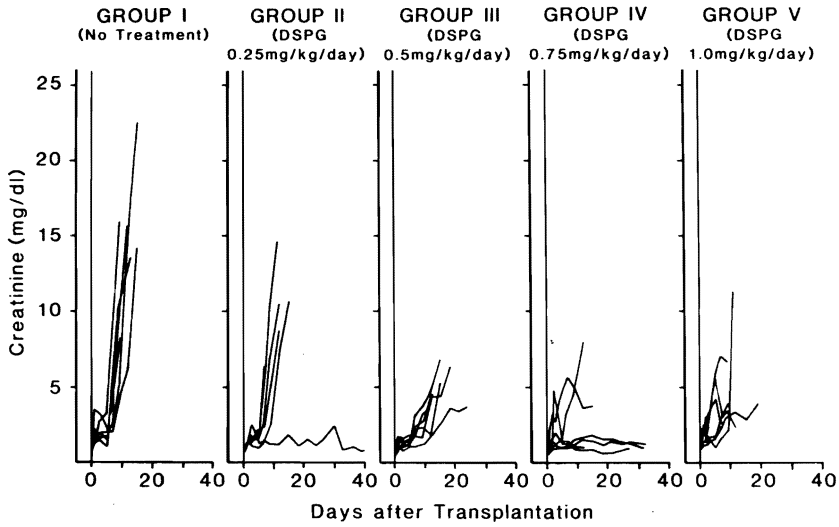

Fig 2. Changes in serum creatinine of KTX dogs treated with different doses of DSPG.

Drug combination. The results were compared with those in group 3 in which 0.5 mg/kg/d DSPG alone prolonged survival from 2 to about 3 weeks. The addition of 5 mg/kg/d CyA with (group 6) or without prednisone (group 7) augmented this effect, but not beyond what could be achieved with CyA alone (Group 10) or CyA plus prednisone (group 9) (Fig 3). Thus, a clean-cut synergistic effect of DSPG with these other agents was not demonstrable.

The best results were with cyclosporine, steroids, and DSPG. Elevation of Cr was well inhibited in this group of animals even though they died during the treatment. Emaciation and diarrhea were prominent in animals that were treated with DSPG for more than 3 weeks. Consequently, the dose of DSPG had to be reduced. Complete histopathologic analyses in animals subjected to the combination study has not yet been completed, but a preliminary observation is that rejection was absent or minimal in dogs under triple drug treatment.

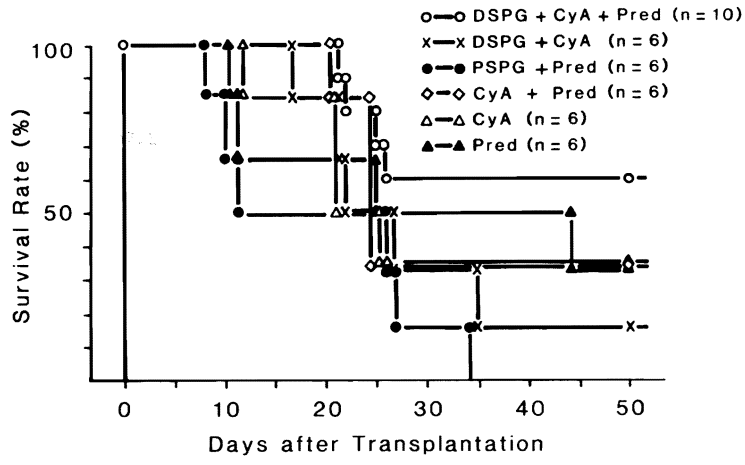

Fig 3. Survival of KTX dogs treated with DSPG and other drugs; DSPG (0.5 mg/kg/d), CyA (5 mg/kg/d), and Pred (5 mg/d).

DISCUSSION

The present study is the first demonstration that DSPG inhibits the rejection of canine kidney homografts. However, the immunosuppressive effect of DSPG alone was feeble and the toxicity was unacceptable. Doses of 0.75 mg/kg/d had a minor therapeutic affect but not enough to yield long animal survival. Doses higher than this caused the dogs to die of lethal emaciation.

Toxicity was somewhat less in rats. However, in contrast to the observation by others,[2,3] most of our rats given higher doses of DSPG died from bronchopneumonia. No pathogen could be identified. The findings were different than those of Ochia et al[3] who reported universal survival of WKA rats for more than 80 days, using the much higher dose of 10 mg/kg/d intramuscularly. Sensitivity of rats to DSPG may differ among strains.

Combination therapy is a potential alternative method with which the toxicity of DSPG could be avoided if a reduced dose would work synergistically with other agents. These hopes were not encouraged by actual experiments. Toxicity of DSPG persisted and the principal immunosuppression was from the CyA, which was an ingredient of the "cocktail." Thus, our results suggest that DSPG alone or in combination probably will not be useful for clinical immunosuppression.

Before pushing ahead with more experiments, it may be wise to learn more of the mechanism of DSPG. DSPG has been said to inhibit the macrophage/phagocyte system instead of affecting lymphocytes.[2] However, there are no well documented experiments to support this contention. Until such evidence is available, further attempts to combine DSPG with other agents are apt to be unrewarding.

SUMMARY

DSPG had a definite but relatively feeble immunosuppressive effect in rats undergoing heterotopic heart transplantation and in dogs after renal transplantation. The drug was toxic in both species, although less so in rats. In dogs, synergistic interactions with cyclosporine and steroids were not evident.

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