

## Efficacy of 15-deoxyspergualin in Canine Lung Transplantation

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Deoxyspergualin (DSG) is a new immunosuppressive agent derived from the bacterium *Bacillus laterosporus*. In this study, we evaluated the immunosuppressive efficacy of DSG in cases of canine lung allotransplantation and the influence of DSG on bronchial anastomotic healing. Twenty-four adult mongrel dogs underwent left lung transplantation. The animals were classified into four groups. Group 1 (n = 4) dogs received DSG dosage of 4.8 to 1.2 mg/kg/day. Group 2 (n = 7) dogs received DSG dosage of 0.8 mg/kg/day. Group 3 (n = 6) dogs were administered a combination therapy consisting of 0.5 mg/kg/day and 10 mg/kg/day of DSG and cyclosporin A (CsA), respectively. Group 4 (n = 7) dogs served as controls, receiving 10 mg/kg/day of CsA. Acute rejection was almost completely suppressed in Group 1 dogs, but severe toxic side effects including emaciation, diarrhea, and pulmonary congestion were often observed. In the grafted lungs of Group 2 dogs, minimal rejection occurred, but improved within three weeks after surgery. A mononuclear cell infiltration characteristically appeared around the bronchus rather than around the vessels. Grafted lung rejection was suppressed sufficiently in Group 3 dogs. However, multiple lung abscesses were found in both the grafted and native lungs of these dogs. *Pseudomonas aeruginosa* was isolated in a bacterial culture study. Side effects such as gastrointestinal disturbances were mild. The bronchial mucosal blood flow in recipient dogs was measured by a laser Doppler velocimetry. The blood flow did not decrease markedly in the dogs of Groups 2 and 4. In contrast, the blood flow of dogs in Groups 1 and 3 was significantly reduced. These data suggest that DSG is an effective immunosuppressive agent in canine lung allotransplantation. However, the DSG dosages for clinical use will require further study. This is especially true for cases of single use or when DSG is used in combination therapy.

### INTRODUCTION

Since cyclosporin A (CsA) was introduced for the use in clinical organ transplantation, lung transplantation has become an increasingly effective modality for the treatment of endstage lung diseases. Nevertheless, lung rejection remains a serious problem. Therefore, it is necessary to develop new immunosuppressive agents and strategies that are more potent and less toxic. Deoxyspergualin

(DSG) is a new immunosuppressive agent derived from the bacterium *Bacillus laterosporus*. In experimental organ transplantation, DSG has prolonged the survival of skin<sup>1)2)</sup>, liver<sup>3)4)</sup>, heart<sup>5)6)</sup>, and kidney<sup>7)</sup> grafts in rats. The effects of DSG on renal allotransplantation in various animals have been evaluated by many researchers. DSG treatment has prolonged graft survival and successfully reversed cases of acute rejection<sup>8)9)10)</sup>. The use of DSG in lung transplantations has been reported previously only once in a series of experimental rat lung transplantations that has been described by Katayama et al.<sup>11)</sup> In this study, determination of the optimal dose of DSG was attempted in canine lung transplantation, evaluating its effectiveness, adverse effects, and influence on bronchial anastomotic healing.

### MATERIALS AND METHODS

Forty-eight adult mongrel dogs of either sex, weighing 8 to 15 kg, were kindly supplied from the animal center for biomedical research of the Nagasaki University School of Medicine and used in this study. A left-sided, single-lung transplantation was performed in 24 dogs using a method similar to that which has been described by Veith and Richards<sup>12)</sup>. The left bronchus was anastomosed with 4-0 prolene continuous sutures, just proximal to the bifurcation of the upper and lower bronchus of the donor lung. Bronchial omentopexy was routinely performed as has been described by Dubois et al.<sup>13)</sup> The dogs were monitored with daily radiographs and weekly blood cell counts. Fiberoptic bronchoscopy was performed every week with the dog under general anesthesia to assess bronchial anastomotic healing. The bronchial mucosal blood flow was assessed using laser Doppler velocimetry. Simultaneously, lung specimenes were obtained during thoracotomy. The extent of acute rejection of the grafted lung was confirmed histologically. Acute lung rejection was classified according to Yousem's classification<sup>14)</sup>. The dogs were housed in the animal facility of the Nagasaki Animal Center. During the study, all animals received care based on the guidelines for animal experiments of the Nagasaki

*Experimental groups*

The animals were classified into four groups as follows:  
 Group 1 (n = 4). After transplantation, the animals were administered intravenous DSG at dosage of 4.8 mg/kg/day for the first two days, 2.4 mg/kg/day for the next two days, and 1.2 mg/kg/day for the following three days. Thereafter, the drug administration was discontinued. (DSG high dose)

Group 2 (n = 7). The animals were treated with intravenous DSG administered at a dosage of 0.8 mg/kg/day for three weeks after the transplantation. (DSG low dose)

Group 3 (n = 6). The animals were administered intravenous DSG and intramuscular CsA, at dosages of 0.5 mg/kg/day and 10 mg/kg/day, respectively, for three weeks after the transplantation. (Combination)

Group 4 (n = 7). The animals were administered intramuscular CsA at a dosage of 10 mg/kg/day for three weeks after the transplantation. (Control)

DSG was supplied from the Nippon-Kayaku Pharmaceutical Co. (Tokyo, Japan).

All recipient dogs received subcutaneous injections of penicillin or cephalosporin during the experimental postoperative period.

Animals suffering from severe diarrhea or anorexia were administered 300 mL/day of subcutaneous or intravenous lactated Ringer's solution.

The dogs were killed within three weeks of the surgery. The lungs and bronchi of these dogs were fixed in 10% neutral formalin, embedded in paraffin, and cut into 5  $\mu$ m thick sections. These sections were then stained with hematoxylin-eosin for light microscopic examination.

*Measurement of bronchial mucosal blood flow by laser Doppler velocimetry (LDV)*

The bronchial mucosal blood flow (MBF) in recipient dogs was measured by a laser flowmeter (ALF 2100, Advance Co, Ltd, Japan). Measurements were made with a probe inserted through a fiberoptic bronchoscope that was applied to the membranous portion of the carina and the first bronchial bifurcation of the transplanted lung. Five blood flow estimations were made at each site. The mean value of these estimations was regarded as the measured blood flow value. It took 10 seconds to obtain each estimation. Measured values were calculated as follows:

$$\frac{L}{C} \text{ ratio} = \frac{\text{MBF at the first bifurcation of the left bronchus}}{\text{MBF at the carina}}$$

To eliminate the influence of acute rejection, only the values for rejection grades 0 and 1 were used.

*Statistics*

Statistical significance was determined by Wilcoxon analysis. A p value less than 0.05 was regarded as significant.

**RESULTS***Survival*

There were no operative deaths. Rejection occurred in only one Group 1 dog during the DSG therapy. However, in the others of Group 1 dogs, rejection occurred within four days after discontinuing the DSG treatment. There was no tolerance or prolonged effects. All dogs became emaciated, and two dogs were anorexic from postoperative day 4 to day 5. Two dogs died of malnutrition on day 7. The remaining two dogs were killed on postoperative days 11 and 14, because of prominent signs of rejection.

Minimal signs of rejection occurred in six of the seven Group 2 dogs. Rejection did not occur in the remaining dog. A severe gastrointestinal disturbance, resulting from DSG administration, also occurred in this group. Anorexia occurred in six of the dogs, and diarrhea occurred in four of the dogs. Five dogs died of malnutrition, intussusception, and thrombosis between days 7 to 15. The remaining two dogs were killed on days 7 and 17.

In four of the six Group 3 dogs, histologic findings consistent with rejection were not found. However, in the remaining two dogs, the finding of minimal rejection was present. Mild malnutrition also was observed in this group. The incidence of anorexia or diarrhea in Group 3 dogs was rare compared with that in Group 1 and Group 2 dogs. Three dogs died of malnutrition between day 7 and 11. Two dogs were killed, one each on days 13 and 16. Only one Group 3 dog survived for 21 days.

Five of the seven Group 4 dogs experienced rejection of their grafted lungs. A CsA dosage of 10 mg/kg was not enough to suppress the allograft rejection in this model. However, the general condition of these dogs was better than that of any other group. Two dogs died because of intussusception and acute rejection between days 8 and 12. Five dogs were killed between days 14 and 21 after surgery (Table 1).

**Table 1.** Results of canine lung transplantation

Group	Grade* of acute rejection	Survival (days)	Cause of death	Side effects	
				Anorexia	Diarrhea
Group 1	0	14	Killed	—	—
	0	11	Killed	+	—
	2	7	Malnutrition	+	—
	0	7	Malnutrition	—	—
Group 2	1	7	Killed	+	+
	1	7	Malnutrition	+	—
	1	15	Malnutrition	+	+
	1	14	Intussusception	+	+
	0	10	Thrombosis	+	+
	1	12	Malnutrition	+	—
	1	17	Killed	—	—
Group 3	0	8	Malnutrition	+	—
	0	7	Malnutrition	+	—
	0	16	Killed	—	—
	1	11	Malnutrition	—	—
	1	13	Killed	—	—
	0	21	Killed	—	—
Group 4	0	8	Intussusception	+	—
	4	12	Rejection	—	—
	1	14	Killed	—	—
	0	21	Killed	—	—
	4	17	Killed	—	—
	0	21	Killed	—	—
	1	21	Killed	—	—

\* last day of drug administration (Yousem's classification)

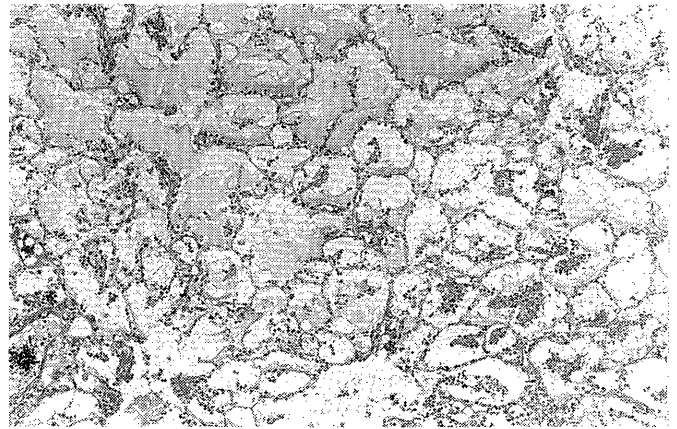
### Histologic examinations of grafted lungs

Signs of pulmonary edema were observed in the lungs of all Group 1 dogs (Fig. 1).

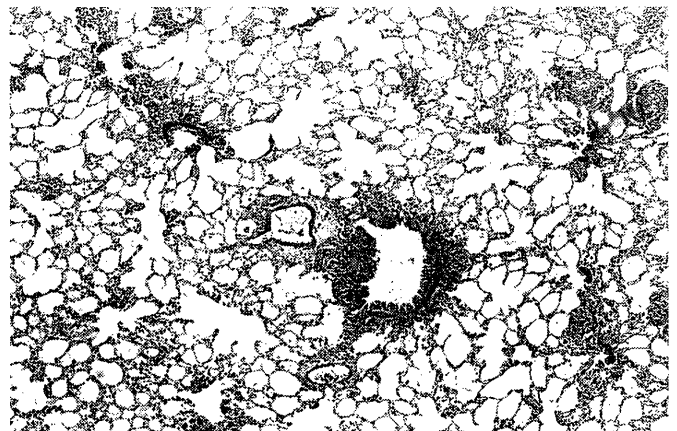
In six of the seven Group 2 dogs, the grafted lungs showed minimal evidence of rejection. However, advanced signs of rejection were not seen. The density of the perivascular or peribronchial cuffing had markedly decreased at the time of death. Mononuclear cell infiltrations were remarkable around the bronchi rather than around the vessels (Fig. 2). Bronchopneumonia was present in two cases.

Rejection did not occur in four of the six Group 3 dogs. However, multinodular lung abscesses were found (Fig. 3). Abscesses were also found in the native lungs, but were not present in the livers, spleens, and kidneys. *Pseudomonas aeruginosa* was isolated in a bacterial culture study. In the remaining two dogs, signs of minimal rejection were present, and a focal mononuclear cell infiltration was sparsely seen at the subpleural, perivascular, and peribronchial sites (Fig. 4).

No remarkable findings were seen in the lungs of Group 4 dogs, except signs of rejection.



**Fig. 1.** Photomicrograph of a grafted lung from a dog in Group 1 on postoperative day 7 (H-E stain, X100). Alveolar edema and bleeding can be observed.



**Fig. 2.** Photomicrograph of a grafted lung from a dog in Group 2 on postoperative day 7 (H-E stain, X 40). A mononuclear cell infiltration is apparent around the bronchus rather than around the vessels.

### Bronchoscopic findings of the bronchial anastomosis

Severe stenosis or dehiscence of the bronchial anastomosis was not observed in any of the groups. In all Group 1 and 3 dogs, the bronchial mucosa adjacent to the anastomosis revealed erythema, edema, and ulcers, consistent with a severe inflammatory response, on bronchofiberscopic examination.

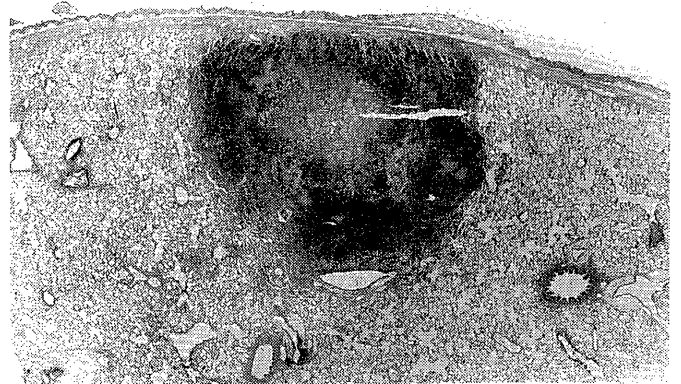
In contrast, the healing of the bronchial anastomosis was superb in all Groups 2 and 4 dogs. Bronchial mucosal inflammation was minimal, with only mild erythema observed, in 60 % of the dogs.

### Microscopic findings of the bronchial anastomosis

Microscopic findings at the site of the bronchial anastomosis in Groups 1 and 3 dogs revealed severe mononuclear and neutrophil cell infiltrations. Ulcers,



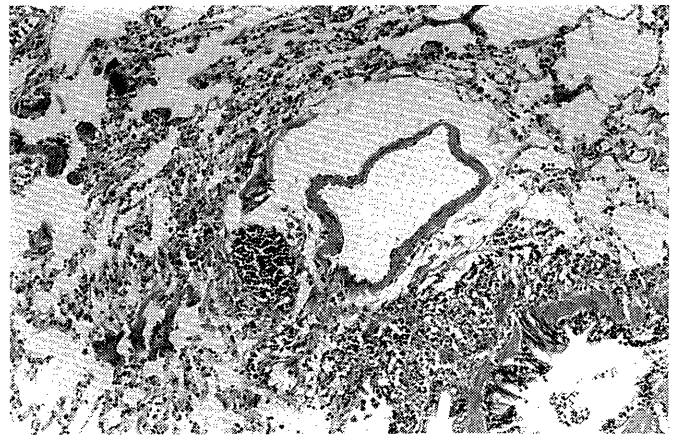
**Fig. 3a.** Photograph of a grafted lung from a dog in Group 3 on postoperative day 7. Multinodular lung abscesses can be seen.



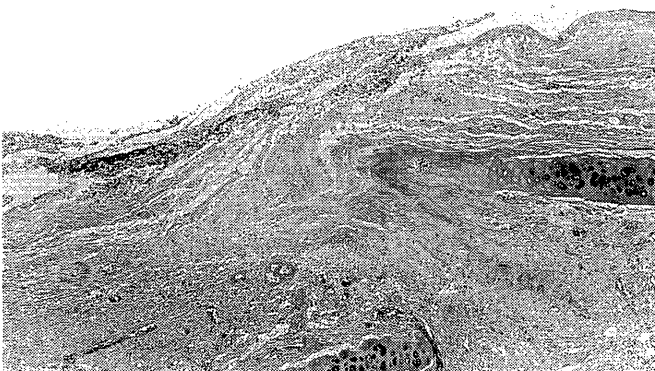
**Fig. 3b.** Photomicrograph of a grafted lung from a dog in Group 3 on postoperative day 7 (H-E stain, X 20). Lung abscesses are visible.



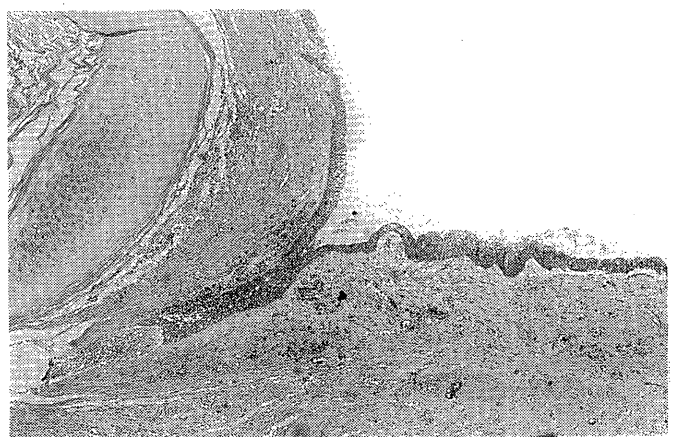
**Fig. 4a.** Photomicrograph of a grafted lung from a dog in Group 3 on postoperative day 7 (H-E stain, X 20). A focal mononuclear cell infiltration can be seen at the peribronchial site.



**Fig. 4b.** Photomicrograph of a grafted lung from a dog in Group 3 on postoperative day 7 (H-E stain, X 100). A focal mononuclear cell infiltration is visible at the perivascular site.



**Fig. 5.** Photomicrograph of the bronchial anastomosis of a Group 3 dog on postoperative day 7 (H-E stain, X 40). Mononuclear cell and neutrophil infiltrations are remarkable. Ulcers, degenerative necrosis, bleeding, and edema also can be observed.



**Fig. 6.** Photomicrograph of the bronchial anastomosis of a Group 2 dog on postoperative day 7 (H-E stain, X 40). Although bleeding and mild edema are present, the epithelium is preserved, and a plasmacyte infiltration dominates.

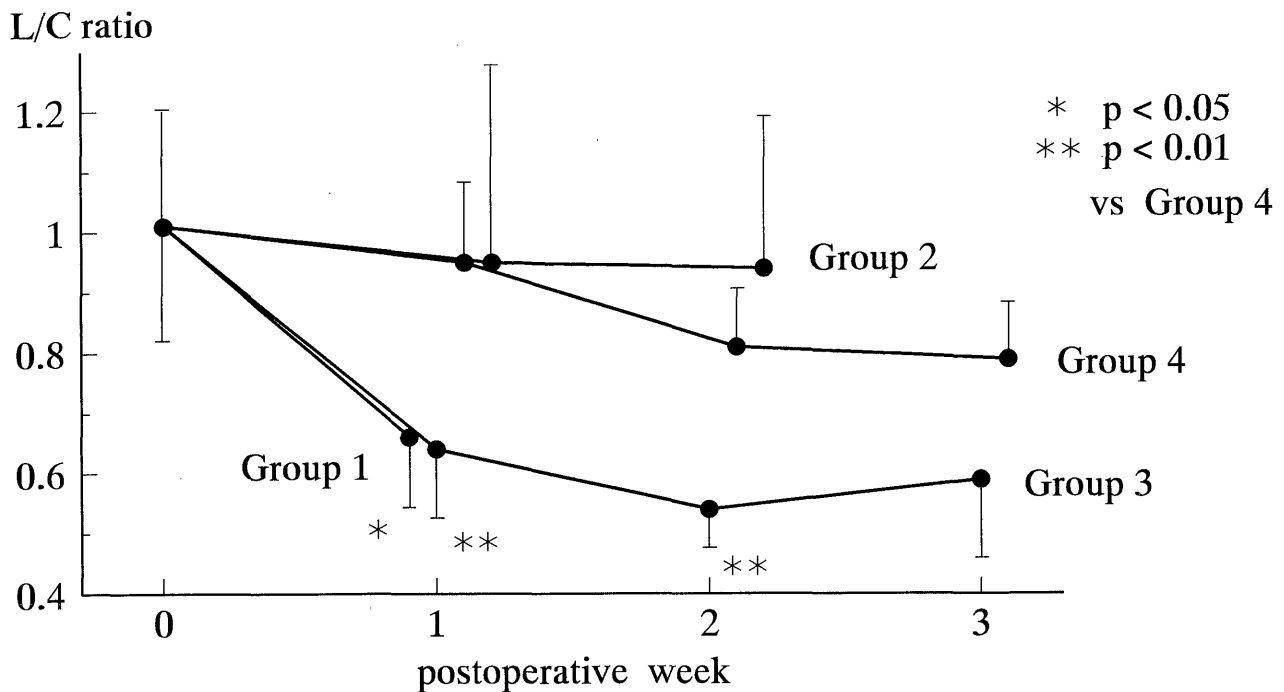


Fig. 7. Bronchial mucosal blood flow measured by laser Doppler velocimetry  
L/C ratio: The blood flow at the left main bronchus / The blood flow at the carina

degenerative necrosis, bleeding, and edema also were noted. Anastomotic healing was retarded (Fig. 5).

The bronchial anastomotic healing went well in Groups 2 and 4 dogs. Although bleeding and mild edema were seen, the epithelium was preserved and there was a dominant plasmocyte infiltration (Fig. 6).

#### Bronchial mucosal blood flow

The bronchial mucosal blood flow of Group 1, described as the L/C ratio, decreased to  $0.66 \pm 0.12$  one week after surgery (Fig. 7).

The L/C ratio of Group 2 was  $0.95 \pm 0.33$  for the first week, and  $0.94 \pm 0.25$  for the second week. Those in Group 3 was  $0.64 \pm 0.12$  for the first week,  $0.54 \pm 0.07$  for the second week, and  $0.59 \pm 0.14$  for the third week. And also those in Group 4 was  $0.95 \pm 0.16$  for the first week,  $0.81 \pm 0.12$  for the second week, and  $0.79 \pm 0.10$  for the third week.

These values for Groups 1 and 3 were significantly lower than those of Group 4 for the first and second week.

#### Complications in DSG-treated dogs

Dose-dependent signs of DSG toxicity included gastrointestinal disturbances (anorexia, diarrhea, and vomiting) and bone marrow depression. The incidence of anorexia was 59%, and the incidence of diarrhea was 24% in all

dogs. Leukocytopenia ( $WBC < 4,000/mm^3$ ) occurred in 38% of the dogs, and thrombocytopenia (platelets  $< 10 \times 10^4$ ) occurred in 71% of the dogs.

#### DISCUSSION

The substance 15-deoxyspergualin (DSG) is a 15-deoxy analogue of spergualin that was isolated from the culture filtrate of *Bacillus laterosporus* in 1981<sup>15)16)17)</sup>. This substance has been reported to have an immunosuppressive effect in various experimental organ transplantation models<sup>18)6)</sup>. The mechanism of action of DSG remains unknown, although it is believed to be different from that of cyclosporine A (CsA) or FK506. DSG suppresses both cellular immunity and humoral immunity<sup>19)</sup>. Therefore, DSG is applied often to xenotransplantation models. In contrast to CsA or FK 506, DSG does not suppress blastogenic responses and the release of IL-1 and IL-2 in response to mitogens. It works to suppress the induction of secondary CTL in vitro and in vivo<sup>20)</sup>. In addition to a different immunopharmacologic mode of action, the most remarkable difference between DSG and CsA is that long-term graft survival can be achieved more easily by initiating DSG administration at the onset of graft rejection rather than by initiating it at the time of transplantation.

DSG has a dose-dependent immunosuppressive effect on dog kidney transplantation. Amemiya et al<sup>8)</sup> have reported that a DSG dosage of 0.6 to 0.8 mg/kg/day is appropriate for beagles with kidney transplantations. This dosage takes into consideration the side effects of the drug, which mainly manifest as gastrointestinal disturbances. Histologic study and laboratory analysis further indicate that 0.6 mg/kg/day of DSG is the critical dosage for immunosuppression. Therefore, we adopted the DSG dosage of 0.8 mg/kg/day. We also administered a higher DSG dosage of 4.8 - 1.2 mg/kg/day which was attempted to reverse ongoing rejection<sup>21)</sup>. Furthermore, the combination usage of DSG and CsA was promoted for lung transplantation. In the combination therapy, DSG and CsA dosages of 0.5 mg/kg/day and 10 mg/kg/day respectively, which are insufficient for a single use, were used.

In this study, rejection was almost completely suppressed by the administration of 4.8 - 1.2 mg/kg/day of DSG, but severe toxicities such as emaciation, diarrhea, and pulmonary congestion were often observed. Therefore, this DSG dosage is considered to be an overdose.

The DSG dosage of 0.8 mg/kg/day had an almost sufficient immunosuppressive effect. Grafted lungs treated with this dosage showed minimal rejection, but the density of perivascular and peribronchial cuffing had markedly decreased when the dogs were killed. Therefore, this dosage appears to be sufficient for maintenance use, but insufficient for early prophylactic use.

Combination therapy with DSG and CsA provided a strong immunosuppressive effect. Side effects such as gastrointestinal disturbances were mild, but serious pulmonary infections were observed. Further studies will be required prior to the clinical use of such combination therapy.

Bronchial anastomotic complications have been a great concern in clinical cases of lung transplantation. Most anastomotic complications are caused by bronchial ischemia and prolonged anastomotic healing that is often affected by an immunosuppressant. Methylprednisolone and azathioprine significantly affect bronchial anastomosis wound healing. However, CsA has advantageous effects on bronchial healing<sup>22)</sup>. Bronchial anastomosis healing depends largely on the regional blood flow. A close relationship has been observed between healing at the anastomotic site and the amount of regional blood flow measured with LDV<sup>23)</sup>. Oikawa has reported that DSG inhibits angiogenesis in a dose-dependent manner<sup>24)</sup>.

In our study, the L/C ratio did not decrease markedly in the Group 2 dogs (0.8 mg/kg/day of DSG) and in the Group 4 dogs (10 mg/kg/day of CsA). The presence of a sufficient blood flow was demonstrated, and bronchial healing was satisfactory. In contrast, the bronchial anastomotic healing was retarded in the Group 1 dogs (initial DSG dosage of 4.8 mg/kg/day) and in the Group 3

dogs (combination therapy with DSG and CsA). In these dogs, the L/C ratio was reduced and the inflammatory response of the bronchial anastomosis was macroscopically and microscopically severe.

The severity of the DSG-induced toxic side effects may vary according to species and may be only mild in human and rats. DSG can be administered to rats at a dosage eight times higher than that in dogs<sup>25)</sup>. It has been reported that DSG administration in renal transplantation patients does not cause serious side effects<sup>26)27)28)</sup>. In our study, the toxicity of DSG was unexpectedly troublesome. In our dogs treated with high dosage of DSG, pulmonary congestion and edema were observed. Ito has reported that interstitial pneumonia, bronchiolitis, and pulmonary edema were observed in dog lungs treated with DSG<sup>29)</sup>.

In conclusion, DSG serves as a strong immunosuppressant in canine lung allotransplantation. However, further dosage studies are necessary prior to its clinical use. This is especially true in the case of combination therapy with CsA and DSG, which can cause severe pulmonary infections, pulmonary congestion, and poor bronchial anastomotic healing.

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