Transpl Int (1994) 7 [Suppl 1]: S449–S452
© Springer-Verlag 1994

Mikio Makabe
Shinichiro Tanaka
Kunzo Orita

The effect of deoxyspergualin (DSG) on rejection and graft-versus-host disease (GVHD) after small bowel transplantation

M. Makabe (✉) · K. Orita
The First Department of Surgery,
Okayama University Medical School,
2-5-1, Shikata-cho, Okayama, Okayama,
700, Japan

S. Tanaka
Department of Surgery,
National Okayama Hospital,
Okayama, Japan

Abstract Both rejection and graft-versus-host-disease may occur after fully allogeneic small bowel transplantation. In this study, we established unidirectional models of rejection and GVHD in rats and evaluated the efficacy of 15-deoxyspergualin (DSG). When F1 small bowel was transplanted into LEW rats (rejection model) the graft was acutely rejected. The administration of DSG (5 mg/kg per day for 10 days) significantly prolonged the survival, but was efficacious only when used prophylactically. When a unidirectional GVHD model (F1 → LEW SBTx) was examined, the administration of DSG from day 0 after grafting greatly suppressed GVHD, resulting in more than 300 days survival. However, only cutaneous GVHD, but not fatal GVHD, was suppressed when the start of administration was postponed until day 4 after grafting. From in vitro studies, DSG inhibited natural killer cell activities to K-562 and skin epidermal cells. The response was well correlated with in vivo GVHD course. These results suggest that DSG is an effective immunosuppressant for both rejection and GVHD when used prophylactically. DSG exerted the effect more stongly against cutaneous GVHD than fatal GVHD by inhibiting natural killer systems.

Key words Deoxyspergualin
Graft-versus-host disease
Small bowel transplantation

Introduction

The success of small bowel transplantation (SBTx) is dependent on the means by which rejection and graft versus host disease (GVHD) are inhibited. Many new drugs have been introduced to prevent both reactions.

Deoxyspergualin (DSG) is known to suppress the rejection of various organs and has previously been used for anti-rejection therapy in clinical transplantions. In the study reported here we established unidirectional models of both rejection and GVHD, and the therapeutic effect of DSG, particularly in relation to the timing of its administration, was examined comparing the two models. Also, the inhibition of immune responses by DSG was studied in vitro.

Materials and methods

Animals

Adult male Lewis ((RT-1^{l}, LEW), WKA (RT-1^{k}) and (LEW × WKA) F1) hybrid rats weighing 300–400 g were used.

Transplantation

A GVHD model and a rejection model were prepared. Auxiliary heterotopic SBTx was performed for the GVHD model by the method of Monchic and Russel [4]. Briefly, a total small bowel was harvested using an aortic cuff and portal vein, followed by perfusion with cold heparinized saline. For revascularization, the graft aortic cuff was anastomosed to the infrarenal abdominal aorta and the graft portal vein was anastomosed to the infrarenal vena cava. The proximal end of the gut lumen was closed and the distal end was opened to the abdominal wall using a Thiry-Vella loop. For the rejection model, orthotopic transplantations were performed in an identical manner except that, following the vascular anastomoses, the small bowel of the host was resected after ligation of the intestinal blood vessels. The duodenum of the host was anastomosed to the jejunum of the graft (4 cm distal from the proximal end), end-to-side. The terminal ileum of the host was anastomosed to the ileum of the graft, end-to-end. Finally, the proximal end of the graft was exteriorized as a jejunostomy.

After surgery the rats were permitted their normal diet and water ad libitum. Fewer than 10% of the animals that expired within 3 days of transplantation were considered to be technical failures and not included in the experimental groups. GVHD and rejection were determined as follows. All animals were inspected daily for clinical signs of GVHD (dermatitis, hyperkeratinosis of foot pads, hair loss, hunched posture) or rejection (diarrhoea, weight loss, hunched posture). Complete rejection in the orthotopic SBTx led to the death of the recipient, thereby giving a clear endopoint to graft/recipient survival.

Chemicals

DSG, a metabolite of *Bacillus laterosporus*, was kindly supplied by Nippon Kayaku, Japan. The drug was dissolved in saline and sterilized by passing through a 0.22 μm filter before use.

Experimental design

Seven groups of rats were studied. Groups I, II and III were the undirectional allogeneic rejection model in which (LEW × WKA) F1 grafts were transplanted into LEW recipients. Group I ($n = 15$) received no immunosuppressive treatment. Group II ($n = 9$) received DSG at 5 mg/kg per day i. m. for 10 days from the day of grafting. Group III ($n = 8$) received DSG at 5 mg/kg per day i. m. for 10 days following the day of onset of rejection (day 4).

Groups IV, V, VI and VII were the unidirectional allogeneic GVHD models in which LEW grafts were transplanted into (LEW × WKA) F1 recipients. Group IV ($n = 22$) received no immunosuppressive treatment. Group V ($n = 7$) received DSG at 5 mg/kg per day i. m. for 10 days from the day of grafting. Group VI ($n = 10$) received DSG at 5 mg/kg per day i. m. for 10 days from day 4 to day 13 after grafting. Group VII ($n = 8$) received DSG at 5 mg/kg per day i. m. for 10 days following the day of onset of GVHD (day 8).

Cytotoxicity

The cytotoxicity assay was carried out by a method described previously [8]. Skin cells were prepared from rat tail by trypsin digestion. LEW, WKA and (LEW × WKA) F1 rat skin cells (10^4 cells/well) labelled with 300 μCi of ^{51}Cr were mixed with spleen cells (10^6 cells/well) from normal and grafted F1 rats, and the mixture was incubated for 12 h at 37 °C in an atmosphere containing 5% CO_2. The cytotoxic activity was expressed as percent cytotoxicity, calculated as follows: percent cytotoxicity = (exp. release − spontaneous release)/(maximum release − spontaneous) × 100. The tumor cell line K562, which was sensitive to natural killer cells and maintained at our laboratory, was also used as target cells in microcytotoxicity assays.

Results and discussion

When immunosuppressants were not used, all LEW rats bearing F1 small bowel died between 7 and 14 days after grafting in the rejection model, showing weight loss and paralytic ileus (group 1). The mean survival (± SD) was 9.7 ± 1.4 days (Fig. 1). F1 rats bearing LEW whole small bowel suffered from skin lesions such as redness of the ears and paws at 7–8 days post-grafting, and died from 12–18 days with fatal GVHD (group IV). The mean (± SD) of survival was 15.8 ± 2.3 days (Fig. 2).

When DSG used prophylactically (from day 0 to 9 after SBTx) in the rejection model (group 2), four out of nine recipients died within 9 days, but the five surviving rats survived more than 20 days, and two of these survived for more than 100 days. In the GVHD model (group V) all rats survived for more than 300 days when DSG was administered from day 0 to 9 after SBTx. Comparing these results, the rejection occurred earlier than GVHD, and DSG treatment was more effective against GVHD. This may be due to the difference in character and volume of effector cells, as target antigens were the same in both the rejection and GVHD models.

In clinical and experimental therapies, DSG is used for anti-rejection therapy [1, 3]. Therefore the effect of the timing of DSG administration was examined. In the rejection model, the host died around 10 days and so

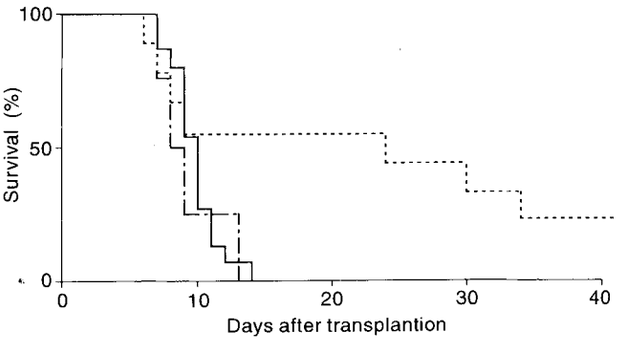

Fig. 1 Host survival after F1 to LEW small bowel transplantation (rejection model). Orthotopic SBTx were performed from F1 to LEW rats. Rejection was diagnosed by host death. DSG (5 mg/kg) was administered intramuscularly as follows: —— no treatment, – – – from day 0 to 9 after SBTx, — · — · — from day 4 to 13 after SBTx

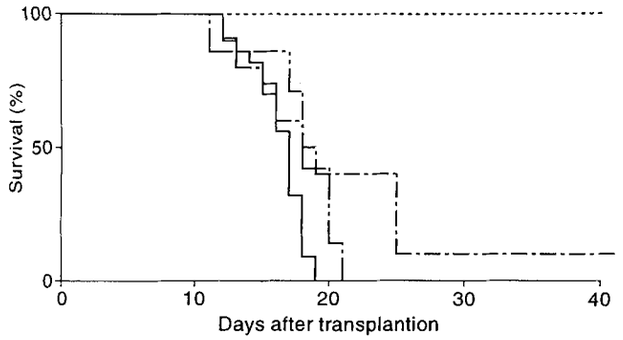

Fig. 2 Host survival after LEW to F1 small bowel transplantation (GVHD model). LEW whole small bowel was transplanted to F1 rats heterotopically. The completion of GVHD was judged by host death. DSG (5 mg/kg) was administered intramusculary as follows: —— no treatment, – – – from day 0 to 9 after SBTx, — - — from day 4 to 13 after SBTx, — - - — from day 8 to 17 after SBTx

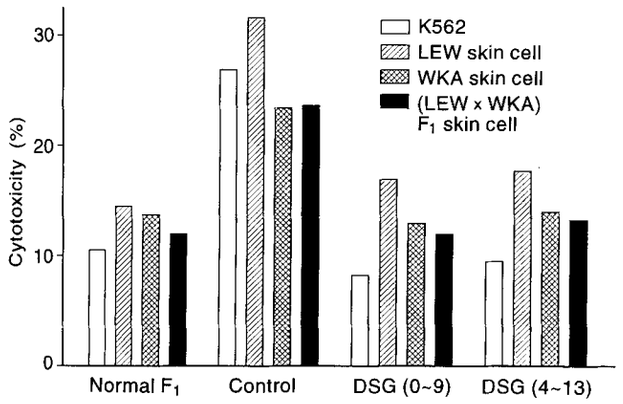

Fig. 3 Cytotoxic activity of host spleen cells on day 7 after LEW to F1 small bowel transplantation: The spleen cells (10^6 cells/well), obtained from normal or LEW to F1 SBTx rats with or without DSG treatment, were incubated with 10^4 cells of K 562 or skin cells for 4 or 12 h, respectively, in U-bottomed microcytotoxicity plates. Data are expressed as percent cytotoxicity

immune reaction leading to rejection may have started 4 days after grafting. When DSG was injected from day 4 to 13 (group III), no prolongation of host survival occurred. These results contradict previous reports. Suzuki et al [6] showed that the survival of rat allogeneic heart grafts was prolonged by DSG treatment and that unresponsiveness in the host was only obtained when DSG administration was started at the time of rejection. Engemann et al. [2] also reported the effect of DSG on acute rejection after rat liver transplantation. The different sensitivity to DSG between small bowel and other organs was left for further examination.

In the GVHD model, DSG was not effective when administered at the onset of cutaneous skin lesions (from 8 days after SBTx, group VII). All rats died from cutaneous and general GVHD. It is possible that GVHD may begin to damage the host before day 8. Therefore, the administration was started at 4 days after SBTx (group VI). Only 1 out of 10 animals survived for 48 days after SBTx (mean survival 18.1 ± 3.2 days). It's interesting that 8 out of 10 rats did not suffer from cutaneous GVHD, possibly indicating that the immune mechanisms provoking cutaneous and general GVHD are different.

We have previously reported [7] that skin epidermal cells are sensitive to naive and alloantigen-sensitized spleen cells in in vitro microcytotoxicity assays and have suggested that skin cells are good targets for both alloreactive T cells and natural killer cells. It has also been shown that cytotoxic activity of spleen cells obtained from SBTx rats in a GVHD model increase corresponding with the onset of cutaneous GVHD [8]. As mentioned above, cutaneous GVHD was well controlled with DSG treatment even when the treatment started just before the onset of cutaneous GVHD (group VI).

As shown in Fig. 3, the cytotoxic activity of host spleen cells determined on day 7 after SBTx was suppressed by DSG. Even when the injection was started on day 4, suppression was observed. These results again suggest that the natural killer system may play a role in GVHD. Nemoto et al. [5] reported the presence of a high activity of alloantigen reactive killer cells in spleens of mice with fatal GVHD after bone marrow transplantation, and little role for natural killer cells. The contradiction may be due to the different species and experimental models used.

In conclusions, DSG is an effective agent for the prevention of rejection and GVHD in rat unidirectional SBTx. DSG had more effect on GVHD than rejection and the effect was exerted only when used prophylactically in these models.

References

1. Amemiya H, Suzuki S, Ota K et al (1990) Transplantation 49:337
2. Engemann R, Gassel HJ, Lafrenz E et al (1988) Transplant Proc 20:237
3. Iwasawa H, Kondo D, Ikeda D et al (1982) J Antibiotics 35:665
4. Monchik GJ, Russel PS (1971) Surgery 70:693
5. Nemoto K, Hayashi M, Ito J et al (1991) Transplantation 51:712
6. Suzuki S, Kanashiro M, Watanabe H et al (1988) Transplantation 46:369
7. Tanaka S, Ota T, Amamiya S et al (1983) Transplant Proc 15:1662
8. Tanaka S, Okada H, Uda K et al (1992) Transplant Proc 24:1161