Rapid communications

Prolongation of graft survival in allogeneic islet transplantation by (–) 15-Deoxyspergualin in the rat

P. K. Walter¹, G. Dickneite³, H. U. Schorlemmer³, H. H. Sedlacek³, S. Jäger¹, G. Feifel¹ and G. Seitz²

¹Departments of Surgery and ²Pathology, University Hospital Homburg/Saar and ³Research Laboratories of Behringwerke AG, Marburg, FRG

Summary. The effect of 15-Deoxyspergualin, a novel drug which has been described to have anti-tumour activity, on allogeneic graft survival (Dark Agouti → Lewis rats) after pancreatic islet transplantation was tested. A marked prolongation of graft survival could be shown using doses of 1.0, 2.5 and 5.0 mg Deoxyspergualin/kg on day 0 until day +9 post transplantation. A maximum of 55.6 days (average) survival time was observed using 2.5 mg/kg Deoxyspergualin compared to 5.2 ± 0.6 days without immunosuppression. Using the

chemiluminescence reaction of recipient monocytes after islet transplantation, a marked suppression of the monocyte system exceeding the treatment period could be observed. Since, in contrast to cyclosporin, B-cell toxicity could not be shown, the new drug seems to be a hopeful step towards successful allogeneic islet transplantation for treatment of diabetes.

Key words: 15-Deoxyspergualin, pancreatic islets, graft survival.

Two major problems have prevented successful allogeneic islet transplantation in human diabetic patients: (1) failure of isolating enough pure islets of Langerhans from a single donor pancreas, and (2) lack of successful management of graft rejection. In experimental allotransplantation of islet grafts across a major histocompatibility complex barrier [1, 2], rejection could not be stopped by conventional immunosuppression, i.e. azathioprine or cyclosporin. Furthermore, there is some evidence for suppression of B-cell function by cyclosporin [3, 4]. Therefore, a new drug with immunosuppressive properties was tested. (–) 15-Deoxyspergualin is a derivative of spergualin which can be isolated from *Bacillus laterosporus* and has a guanidinic-like structure. Its anti-tumour activity was first described by Iwasawa et al. [5]; previous studies [6] have demonstrated that Deoxyspergualin prolongs allogeneic skin graft survival and survival of allo-transplanted hearts and kidneys [7]. After allogeneic kidney transplantation, an indefinite graft survival has been shown during treatment with only 10 injections of deoxyspergualin [7]. Therefore, it seemed reasonable to test the effects of this substance after allogeneic islet transplantation.

Materials and methods

Male Lewis rats weighing 200 to 250 g and male DA (Dark Agouti) rats weighing 170 to 190 g were purchased from Zentralinstitut für Versuchstierzucht, Hannover, FRG. Streptozotocin was purchased

from Sigma Chemicals, Munich, FRG. (–) 15-Deoxyspergualin was a gift from Dr. Umezawa, Institute of Microbial Chemistry, Tokyo, Japan. In order to prevent allogeneic graft rejection 15-Deoxyspergualin was dissolved in sterile phosphate-buffered saline (PBS, pH 7.2) and injected intraperitoneally 1.0, 2.5 and 5.0 mg/kg on days 0–9 after transplantation. As a defined rejection model, the system DA (Dark Agouti) – islets → diabetic Lewis (LEW) rats, RT¹ → RT¹ was used. Streptozotocin (STZ) diabetic LEW rats (duration of diabetes at least 2 weeks) received 1000 islets freshly isolated from 2 DA rats (modified collagenase method as previously described, 8, 9), each by intraportal injection. Diabetes was induced by injecting 65 mg STZ/kg intravenously. None of the control animals ($n=10$) had spontaneous reversal of diabetes and died within 4 weeks with the symptoms of weight loss, hyperglycaemia, diarrhea and polyuria. Postprandial blood glucose levels were measured daily using the test combination “Glucoquant Glucose”, Boehringer Mannheim, FRG. This method is based upon glucose phosphorylation by hexokinase.

Diabetic control rats ($n=10$) were transplanted with 1000 allogeneic islets using saline injections instead of immunosuppressant. Rejection of transplanted islets was assumed when blood glucose of the recipients rose again above 10 mmol/l.

Chemiluminescence reaction: two millilitres of heparinized peripheral venous blood was collected by puncture of the retro-orbital vein plexus of rats. Monocytes were separated by density gradient centrifugation (800 g, 20 min) over Ficollhypaque (density 1.077 g/l). Monocytes were suspended in tissue culture medium (TCM), 199 containing penicillin, streptomycin (100 U/ml each) and heparin (10 U/ml) [10]. Monocytes were stimulated by the addition of immune complexes (tetanus toxoid-anti tetanus toxoid, formed at an equilibrium). The chemiluminescence reaction was performed in a Biolumat (model LB 9505, Berthold Co., Wildbad, FRG) in the presence of 20 µg/ml luminol in polystyrene vials (4×10^6 monocytes/ml). The integral of relative light units (RLU) was calculated after 15 min, and represented the sum of light emission caused by the O₂-radicals during this period.

Table 1. Islet transplantation across a major histocompatibility complex barrier: prolongation of graft survival by 15-Deoxyspergualin

Group	Graft survival (days)	Mean $\pm$ SEM
1 Control (phosphate buffer solution) (n = 10)	3, 7, 2, 5, 5, 8, 7, 3, 6, 6	5.2 $\pm$ 0.6
2 Deoxyspergualin (1 mg/kg) (n = 5)	8, 15, 20, 29, 65	27.4 $\pm$ 10.0 ($p < 0.002$)
3 Deoxyspergualin (2.5 mg/kg) (n = 10)	67, 23, 22, 8, 12, 11, 15, 93, 132, 173	55.6 $\pm$ 18.6 ($p < 0.002$)
4 Deoxyspergualin (5 mg/kg) (n = 5)	16, 50, 13, 15, 17	22.2 $\pm$ 7.0 ($p < 0.002$)

Table 2. Chemiluminescence reaction^a of recipient monocytes after islet transplantation across a major histocompatibility complex barrier

Days after transplantation	Control	15-Deoxyspergualin ^b
0	1983 $\pm$ 236	2072 $\pm$ 272
9	2126 $\pm$ 213	662 $\pm$ 262
13	1837 $\pm$ 287	537 $\pm$ 76
16	1987 $\pm$ 199	713 $\pm$ 175
20	2013 $\pm$ 237	660 $\pm$ 247
27	2135 $\pm$ 66	1394 $\pm$ 327
37	2268 $\pm$ 134	1730 $\pm$ 376
40	2188 $\pm$ 259	2208 $\pm$ 164

^a + immune complex (IC) in vitro, relative light units (RLU)/15 min $\times 10^3$; ^b 1 mg/kg intraperitoneally (i.p.) day 0–9

Statistical analysis

For statistical analysis the two tailed Mann - Whitney U-test was used. A p value of < 0.01 was considered statistically significant.

Results

Table 1 shows a marked prolongation of graft survival of allogeneic transplanted islets during treatment with different doses of 15-Deoxyspergualin. All 3 concentrations (1.0, 2.5 and 5.0 mg/kg, injected up to 10 times after transplantation) were effective ($p < 0.002$ compared to the saline group).

The maximum of allo-graft prolongation is found at 2.5 mg/kg (55.6 $\pm$ 18.6 days).

In Deoxyspergualin-treated recipients of allogeneic islets, a rapid normalization of blood glucose can be observed on the first day post transplantation. Whilst in a cyclosporin-treated control group a transient increase in blood glucose occurs during the first week after transplantation, there is no such phenomenon in the Deoxyspergualin-treated group (data not shown). Monitoring the activity of blood monocytes during the transplantation experiments, a marked suppression of monocytic

activity in the drug-treated (Deoxyspergualin) group was observed. This was demonstrated by immune complex mediated stimulation of oxygen derived free radical production in the Deoxyspergualin-treated animals (1 mg/kg) compared with the control group (Table 2). The suppression of the monocyte activity lasted far beyond the application of the drug until day 37 (Table 2).

Histologic examination of livers embolized with allogeneic islets revealed normal islet structures during immunosuppressive treatment with Deoxyspergualin (2.5 mg/kg).

Discussion

(-) 15-Deoxyspergualin seems to be a potent drug in preventing rejection of allogeneic transplanted islets of Langerhans in the rat. The mechanism of its action has not yet been completely elucidated. While it strongly suppressed the monocyte system, no effect on T-lymphocytes could be demonstrated [6, 11]. Interestingly, there seems to be a dose-dependent optimum of immunosuppression which is shown to be about 2.5 mg/kg. Higher and lower doses both lead to a decrease in prolongation of graft survival. In skin transplantation (Fisher $\rightarrow$ LEW), however, the most effective dosage was 1.0 mg/kg [6].

The presented data show that 15-Deoxyspergualin has the potency to inhibit the generation of O_2 -radicals in monocytes. We recently demonstrated [11] that Deoxyspergualin also inhibits other monocyte functions such as the secretion of hydrolytic enzymes and the expression of class II antigens on splenic macrophages.

Thus, 15-Deoxyspergualin may represent an interesting drug which has a remarkable potency of immunosuppression and prolongation of graft survival in allogeneic islet transplantation.

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Dr. Paul Walter
Department of Surgery
University Hospital
D-6650 Homburg/Saar
FRG