Short Communication

The Effect of Gemcitabine in the Treatment of Rejection in Experimental Small Intestine Transplantation

(small bowel transplantation / acute rejection / gemcitabine / immunosuppression / rat)

M. KUDLA, J. KŘÍŽ[1], D. JANOTOVÁ[2], P. GIRMAN[1], H. MERGENTAL, M. KOČÍK, E. HONSOVÁ[2], M. ADAMEC

Department of Transplant Surgery, [1]Centre of Diabetes, and [2]Department of Pathology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Abstract. The aim of our study was to test the immunosuppressive effect of gemcitabine in monotherapy following heterotopic SBT in the rat. The BN and LEW rats were used as donors and recipients, respectively. Recipients were divided into 4 groups – group A without immunosuppression, group B treated with a therapeutic dose of tacrolimus, groups C and D treated with various doses of gemcitabine (100 and 150 μg/kg/day). Immunosuppression was administered once a day for 7 days after SBT, when the animals were sacrificed and a histological examination of grafts was performed. Only in group B no signs of acute rejection were seen. Significant differences ($P < 0.01$) were noted only between group B versus groups A, C, and D. No significant differences were demonstrated between groups A versus groups C, D and between group C versus group D. Monotherapy by gemcitabine (when administered at given doses) was not shown to be effective in preventing acute rejection in a rat model of heterotopic SBT.

Despite the advances made in parenteral nutrition, small bowel transplantation (SBT) may be a life-saving method in indicated patients (Goulet et al., 2000). However, introduction of the method as a standard therapeutic procedure into a broader clinical practice has been prevented by poor outcomes compared with those reported for other transplant organs. One of the main causes is difficult-to-treat rejections in spite of calcineurin inhibitor-based immunosuppressive regimen (Grant, 1997).

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Corresponding author: Michal Kudla, Department of Transplant Surgery, Institute for Clinical and Experimental Medicine, Videňská 1958/9, 140 21 Prague 4, Czech Republic. Tel.: (+420) 261 364 105; Fax: (+420) 261 362 822; e-mail: michal.kudla@centrum.cz

Abbreviations: BN – Brown Norway, dFdC – 2'2'-difluorodeoxycytidine, LEW – Lewis, SBT – small bowel transplantation.

Several recently published experiments have explored the effect of gemcitabine (2'2'-difluorodeoxycytidine, dFdC) using models of allogenic heart, kidney, and liver transplantation. Although liver grafts failed to engraft when using therapeutic gemcitabine doses (Mergental et al., 2005), heart and renal graft rejection was prevented by gemcitabine with an efficacy comparable to that of cyclosporine A (CyA) and tacrolimus (Margreiter et al., 1999; Jung et al., 2002; Jeske et al., 2003). The promising uses of gemcitabine in heart and renal transplantation made us test the drug in a model of heterotopic SBT in the rat.

Material and Methods

Animals

Adult inbred males of the Brown-Norway (BN) RT1n strain, weighing 160–310 g were used as donors while inbred males of the Lewis (LEW) RT1l strain, weighing 200–380 g served as recipients (Charles River, Sulzfeld, Germany). All experiments related to these animals were performed according to the protocols reviewed and approved by the institutional Animal Care and Use Committee. Twelve hours prior to surgery, food was made unavailable to rats. Water was freely available until the procedure.

Groups

Animals were divided into four groups: A (N = 6) BN→LEW without immunosuppression; B (N = 6) BN→LEW treated with a therapeutic dose of tacrolimus (Fujisawa, GmbH Munich, Germany, 1 mg/kg/day i.m.); C (N = 6) BN→LEW treated with gemcitabine (Gemzar, Lilly France S.A., Suresnes, France, 100 μg/kg/day s.c.); D (N = 6) BN→LEW treated with gemcitabine (150 μg/kg/day s.c.).

SBT

We used a modified model of heterotopic SBT with portocaval venous drainage (Monchik and Russel,

1971). From the animals under general anesthesia ketamine i.m. 10 mg/kg (Narkamon 1%, Leciva, Prague, Czech Republic) + chlorpromazine i.m. 2.5 mg/kg (Plegomasin 0.5%, Egis Pharmaceuticals Ltd., Budapest, Hungary), 7–10 cm of the proximal jejunum with the extrahepatic section of the portal vein and superior mesenteric artery were removed. The graft was perfused *in situ* using 3 ml 4°C cold heparinized saline (100 IU heparin/ml) and implanted to the recipient after a short period of ischemia (27–87 min). Anastomoses were created using continuous aorto-aortic and porto-caval end-to-side suture (Nylon 9-0). The proximal end of the intestine was closed blind, with the distal end used as stomy in the right mesogastrium. Manipulation time ranged from 29 min to 47 min. The technical success of SBT was determined by the presence of mesenterial vessel pulsation, good graft perfusion and survival of animals for more than 3 days.

Postoperative care

Following the procedure, the animals were placed in a heated box with immediate access to water. The animals received, beginning the next postoperative day, standard laboratory chow. The animals' weight was checked daily as was their health condition. On postoperative day 3, a 1-cm section of donor intestine was removed under general anaesthesia for histology. At the same time, the stomy was flushed with saline to prevent its obstruction. The animals were sacrificed on day 7 to remove donor and recipient intestine samples for histology.

Histology

Specimens of the whole bowel wall were fixed in 10% neutral formalin solution and passed according to the routine protocol. The 5-μm cut sections were stained with haematoxylin and eosin. Three degrees of rejection were determined dependent on the combination of inflammation and crypt injury (McDiarmid et al., 1994, Table 1).

Statistical analysis

The χ^2 test was used for statistical evaluation.

Results and Discussion

Only animals with technically successful SBT were included into analysis.

In group A, no signs of rejection were seen on day 3, while on day 7 all animals showed signs of rejection of mild (N = 2), moderate (N = 2) and severe (N = 2) grade. In group B, mild rejection was noted on day 3 in two cases while no signs of rejection were reported on day 7. In group C, there were no signs of rejection on day 3, with mild rejection in one animal, moderate rejection in four animals, and severe rejection in one animal on day 7. In group D, mild rejection was seen in two cases on day 3, with mild and moderate rejection in one and five cases on day 7, respectively. Significant differences ($P < 0.01$) were seen only between group B versus groups A, C, D. No significant differences were noted between group A versus groups C, D, and between groups C and D (Table 2).

No pathological changes in bowel morphology were demonstrated in the actual recipient's intestines.

Gemcitabine, pyrimidine antimetabolite originally developed as a virostatic agent, is phosphorylated by lymphocyte deoxycytidine kinase to di- and triphosphate metabolites. These active metabolites block DNA synthesis in lymphocytes, impair their proliferation, which subsequently results in the immunosuppressive effect (Plunkett et al., 1989; Plunkett et al., 1995).

A study carried out by Margreiter et al. reported, in an *in vitro* study, that the antiproliferative effect of dFdC depends on intra-lymphocyte concentration. In an *in vivo* model of heart transplantation, prolonged cardiac graft survival rates were obtained at a dose of 100 μg/kg/day. However, doses higher than 125 μg/kg/day did not improve graft survival. Administration of higher doses (150, 300, 600, 6000 μg/kg/day) led to death of

Table 1. Grading of rejection

	Degree of rejection	Histological finding
Degree 0	No signs of rejection	No presence of inflammatory cells in the lamina propria and interepithelially
		No signs of cryptitis
		No changes in villous architecture
Degree 1	Mild rejection	Presence of inflammatory cells in the lamina propria
		No epithelial changes
		Mild cryptitis
		No changes in villous architecture
Degree 2	Moderate rejection	In between degrees 1 and 3
		More marked presence of inflammatory cells
		Superficial changes in villous architecture
		Epithelial destruction involving up to a half of the vile
Degree 3	Severe rejection	Transmural infiltration with lymphocytes and other elements
		Major epithelial destruction involving more than a half of the vile

Table 2. Histological findings of acute rejection in experimental groups

	Immunosuppression	Histological signs of rejection		
		Day 3	Day 7	P
A	No IS	0, 0, 0, 0, 0, 0	1, 1, 2, 2, 3, 3	
B	Tacrolimus 1mg/kg/d	0, 0, 0, 0, 1, 1	0, 0, 0, 0, 0, 0	P < 0.01 vs. A, C, D
C	dFdC 100 μg/kg/d	0, 0, 0, 0, 0, 0	1, 2, 2, 2, 2, 3	ns vs. A, D
D	dFdC 150 μg/kg/d	0, 0, 0, 0, 1, 1	1, 2, 2, 2, 2, 2	ns vs. A, C

0 – No signs of rejection; 1 – Mild rejection; 2 – Moderate rejection; 3 – Severe rejection; IS – immunosupresion.

animals with functional grafts. The immunosuppressive effect of dFdC was later documented by Jung, who used dFdC in a model of acute heart and kidney rejection in the rat. The effective dose in the model of heart transplantation was 130 μg/kg/day and 150 μg/kg/day in a model of kidney transplantation.

In our experiments the used regimen was based on the dosage reported in the two above studies. Neither the low dose of dFdC (100 μg/kg/day) nor the high one (150 μg/kg/day) prevented rejection changes. In spite of that, we preferred not to further increase the dose, given the repeatedly reported toxicity of the agent. While gemcitabine was unable to inhibit the development of acute intestinal rejection in any of the therapeutic schemes used, tacrolimus was able to completely block intestinal graft rejection. Although we did not monitor tacrolimus blood levels, the dose was used according to the previously reported rat model of allogeneic islet transplantation (Ricordi et al., 1995)**.**

Our results suggest that gemcitabine at the doses used will not inhibit rejection in a fully allogeneic rat model. Potentially more convenient combination therapy using calcineurin inhibitors (CyA, tacrolimus) and gemcitabine, shown to be effective in a model of heart transplantation, will be tested in future experiments using a model of SBT.

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