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The use of FK506 and RS61443 for reversal of small-bowel rejection

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Successful clinical small-bowel transplantation is still difficult to achieve [3, 6]. Two features render the small intestine unique among vascularised solid organ grafts. First, the bowel contains a large amount of lymphoid tissue within the Peyer's patches, mesenteric lymph nodes, and intraepithelial lymphocytes, which are thought to mediate graft-versus-host disease and provide a major stimulus for the recipient's immune system [10]. Unfortunately, mere surgical reduction of these tissues, by using segmental allografts, does not furnish any immunological advantage [12]. Second, the small bowel lacks specific serum markers such as blood urea nitrogen (BUN) in the kidney or bilirubin in liver transplantation. Clinical signs such as fever, pain, or tenderness of the abdomen may indicate an already advanced destruction of the graft. Therefore, very potent immunosuppressive regimens are necessary to avoid small-bowel allograft rejection or even to reverse an ongoing rejection process. Cyclosporin was shown in small and large animal models to control rejection reactions sufficiently [4, 13]. However, there are two even more promising immunosuppressive agents currently under investigation. FK506, a macrolide lactone isolated from *Streptomyces tsukubaensis,* leads to long-term survival of small-bowel allografts in a rodent model and has already been used in a few clinical small-bowel transplantations [11, 14]. RS61443, a mycophenolic acid morpholinoethylester, selectively inhibits T- and B-cell proliferation [9]. We have investigated the use of FK506 and RS61443 for the reversal of small-bowel allograft rejection in a small animal model.

Key words: FK506 – RS61443 – Small-bowel rejection

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Methods

The experimental *animals* were adult male rats of the inbred Lewis (LEW) ($RT1^l$) and Brown Norway (BN) ($RT1^n$) strains, weighing from 180 to 280 g. In each instance, BN rats served as donors and LEW rats as recipients of grafts.

The *operative procedure* involved the orthotopic transplantation of the entire small bowel, with end-to-side anastomoses of the superior mesenteric artery and the portal vein of the graft to the infrarenal aorta and vena cava, respectively, of the recipient. After revascularization of the graft, the small bowel of the recipient was resected to an extent equivalent to the length and type of the allograft. The allograft was interposed by means of two end-to-end intestinal anastomoses.

Five *experimental groups* were formed according to the different immunosuppressive regimens. All animals received a basic immunosuppression with cyclosporin A (CyA; 10 mg/kg) for the first 5 postoperative days. In groups 2–5 all animals were treated from day 13–15 postoperatively with CyA, FK506, or RS61443. Biopsies were taken from each animal on days 13, 16, and 25 postoperatively and at sacrifice.

Group 1 ($n = 13$) animals did not receive any further immunosuppressive therapy.
Group 2 ($n = 11$) recipients received CyA again on days 13–15 postoperatively as a dose of 10 mg/kg.
Group 3 ($n = 9$) rats were treated from day 13–15 postoperatively with a dose of FK506 2 mg/kg.
In group 4 ($n = 3$), a rescue therapy was started on day 13 with 20 mg/kg RS61443 and stopped on day 15.
In group 5 ($n = 9$) RS61443 was administered in a dose of 40 mg/kg on the same days as in group 4.

Determination of rejection. Complete rejection of the allograft was easily determined because it led to the death of the recipient in each instance.

Graft-versus-host reaction (GvHR). All animals were monitored daily for clinical signs of GvHR (redness of ears, snout, and paws, hair loss, diarrhea). Spleen, lymph nodes, liver, and host intestine were examined histologically at the time of autopsy or sacrifice.

Skin grafting. Full-thickness skin grafts, 2.5 cm^2, of the abdominal wall [2] from homozygous donor animals were prepared. These were then sutured into position on the recipient's neck using an interrupted 4/0 silk suture. The grafts were inspected daily, and rejection was declared when more than 50% of the graft surface had become scabby or necrotic.

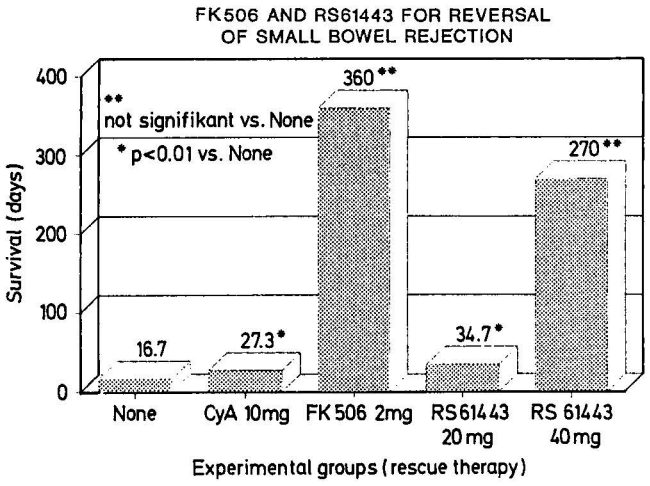

Fig. 1. Survival times of small-bowel allograft recipients after rescue therapy with cyclosporin A (CyA), FK506, or RS61443

Fig. 3. Mesenteric lymph node from a transplanted small intestine on postoperative day 13 (see text)

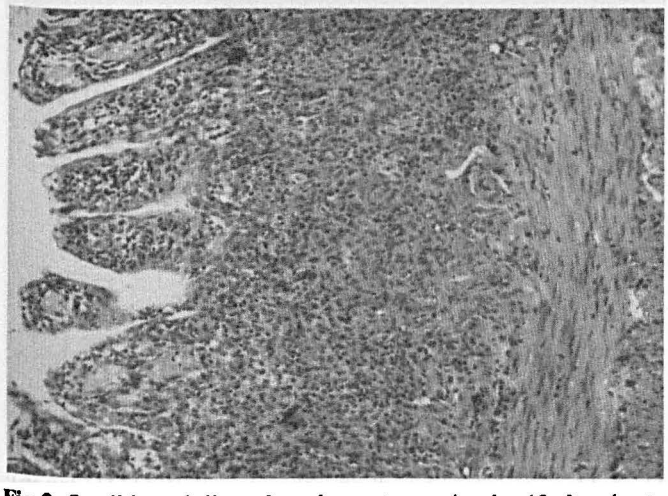

Fig. 2. Small-bowel allograft on the postoperative day 13 after short-term CyA therapy (see text)

Histology examination. The small-bowel graft with its mesentery and lymph nodes as well as the recipient's own mesenteric lymph nodes, spleen, and liver were obtained at the time of autopsy or at the time of sacrifice. The specimens were fixed in formalin and prepared for light microscopy study with H & E staining.

Statistical analysis. All results were expressed as mean and SEM. Student's t-test was performed for the calculation of P values.

Results

Animals that received only the basic immunosuppression regimen with CyA (10 mg/kg) for 5 days died from rejection reaction after an average of 16.7 ± 1.7 days. Recipients receiving an immunosuppressive therapy with either CyA (10 mg/kg) or RS61443 (20 mg/kg) from day 13–15 postoperatively in addition to the basic immunosuppression showed a prolonged survival. But finally all animals died from rejection on day 27.3 ± 4.8 in the CyA group or day 34.7 ± 1.5 in the RS61443 (20 mg) group (Fig. 1). The difference was not statistically significant in the CyA group ($P > 0.05$), but it was in the RS61443 group ($P < 0.05$). All rats in groups 3 and 5 which received a rescue therapy with either FK506 (2 mg) or RS61443 (40 mg) showed an indefinite survival ($P < 0.01$). Animals that were sacrified after more than 360 days (in group 3) or 270 days (in group 5) revealed no signs of a rejection reaction.

Pathohistology study

The biopsy studies taken on the 13th postoperative day showed a thickening of the small-bowel wall in all animals and a fibrotic encapsulation of the mesenteric lymph node. Histology sections of the transplanted small bowel revealed a severe lymphocyte infiltration into the lamina propria and a shortening and blunting of the villi. In addition, there was an almost complete loss of goblet cells in the villous epithelium (Fig. 2). In the mesenteric lymph nodes we found a follicular hyperplasia with an increased number of germinal centers and a sinus histiocytosis (Fig. 3). After a 3-day treatment with either FK506 or RS61443, the histology sections taken on the 25th and 36th day, respectively, showed an almost complete recovery of the small bowel and lymph node architecture (Figs. 4–7).

Graft-versus-host disease

None of the animals showed clinical signs of GvHR. This was the case before the animals received rescue therapy as well as thereafter. Examinations of host tissue (spleen, lymph nodes, liver) on days 13, 16, 25, and at sacrifice revealed no histological signs of GvHR such as loss of architecture, depletion of lymphoid cells, or periportal lymphocytic infiltration.

Skin grafts

Three animals from groups 3 and 5 received a skin graft more than 270 days after small intestine transplantation and cessation of immunosuppressive therapy. No clinical

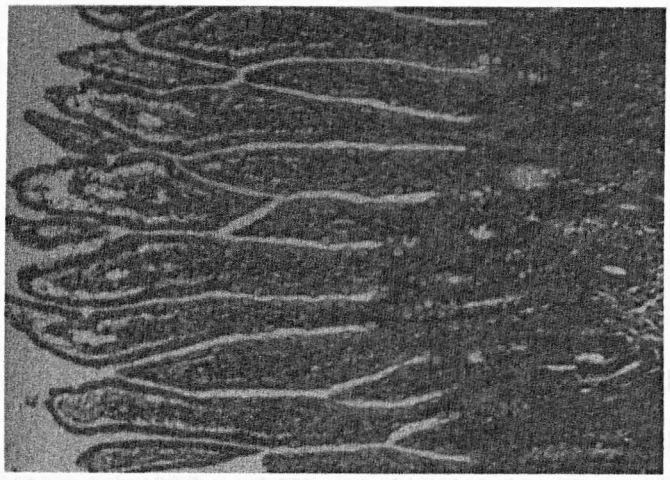

Fig. 4. Small intestine after rescue therapy with FK506 (25th day); regular height of the villi with only little edema and cellular infiltration

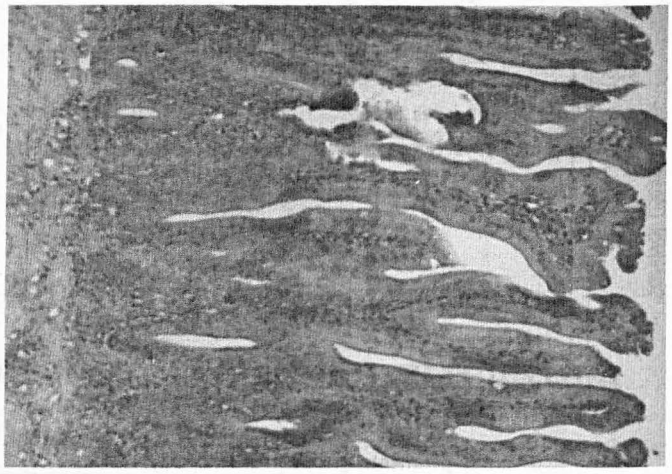

Fig. 6. Small-bowel allograft after rescue therapy with RS61443 (day 36); normal length of the villi with only minor cellular infiltration

Fig. 5. Mesenteric lymph node on the day 25 after FK506 treatment; slight sinus histiocytosis and dispersed architecture

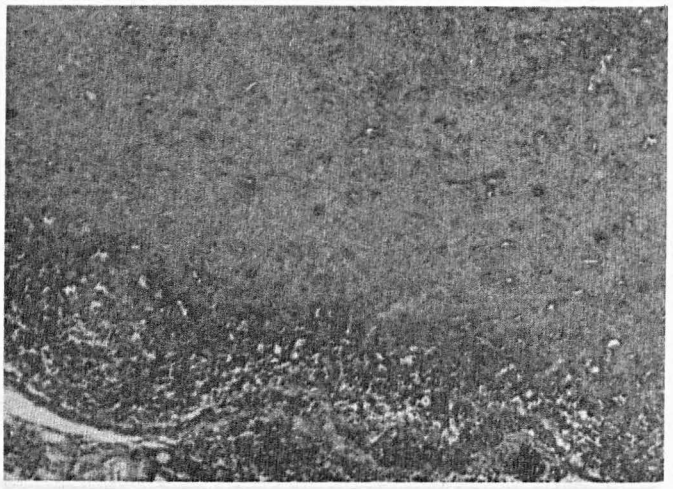

Fig. 7. Mesenteric lymph node after therapy with RS61443 (day 36); regular architecture with no signs of rejection reaction

or histological signs of rejection were seen at the time of skin grafting. All skin grafts were rejected between day 10 and 14 after transplantation. However, this rejection reaction had no influence on the function, integrity, or histological appearance of the small-bowel graft.

Discussion

The new immunosuppressive drugs FK506 and RS61443 have increased the hope that small-bowel transplantation will become a clinical reality in the near future. FK506 acts similarly to CyA and inhibits the production of interleukin (IL-2, IL-4, IL-5), interferon (IFNτ), and tumor necrosis factor (TNFα) from stimulated mononuclear cells [1]. Studies in rats have shown that FK506 inhibits lethal, acute GvHR [7] and leads to indefinite survival of small-bowel transplant recipients after a short course of treatment [11]. RS61443, a semisynthetic derivative from mycophenolic acid, inhibits inosine 5'-monophosphate dehydrogenase and guanosine monophosphate synthetase and thereby specifically depresses the "de novo" synthesis of nucleic acid [5]. As stimulated T- and B-lymphocytes depend on this "de novo" pathway, RS61443 inhibits their activation. RS61443 prolongs graft survival in experimental heart transplantation [9].

In our study we have tested both drugs for their ability to reverse an ongoing rejection reaction. All animals receiving either FK506 or RS61443 on day 13 postoperatively showed a destruction of the mucosa, a thickening of the small-bowel wall, and a marked cellular infiltration. Just a 3-day treatment with FK506 and RS61443 completely abrogated the rejection process and led to an indefinite survival of all recipients. None of the animals showed any clinical side effects, such as retching, tremor, or decrease of spontaneous mobility [8]. However, the animals receiving RS61443 lost weight and suffered from infectious complications approximately 20 days after cessation of the treatment. None of the recipients died from these events, and clinical integrity could be restored by treatment with antibiotics. The rejection of all skin grafts without any influence on the transplanted small bowel indicates that FK506 and RS61443 produce a status of specific tolerance. The ability of the immune system to act

against a new stimulus seems to be unaffected. Todo et al. [14] reported on 5 cases of successful clinical small-bowel transplantation using FK506. Four of these patients received a small-bowel allograft together with a liver; the protective potential of a liver transplant on a second graft, especially with a venous outflow into the portal vein, is still not clear. Nevertheless, these results are encouraging regarding the future of clinical small-intestine transplantation. In our model we have demonstrated the efficiency of FK506 and RS61443 to reverse an ongoing rejection reaction with an already destroyed graft architecture. These results are promising for the treatment of severe and otherwise uncontrollable rejection reactions and possibly even for the treatment of chronic rejection.

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