World Journal of Gastroenterology

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DOI: 10.3748/wjg.v2.i1.36

World J Gastroenterol 1996 March 25; 2(1): 36-40
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CLINICAL ARTICLES

Anti-rejection therapy with tripterygium woifordii and low-dose cyclosporine for small bowel transplantation in pigs

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Supported by The National Natural Science Foundation of China, No. 1991069.

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Received: November 18, 1995
Revised: January 13, 1996
Accepted: February 1, 1996
Published online: March 25, 1996

Abstract

AIM: To determine whether anti-rejection therapy with tripterygium wolfordii (TW) and low-dose cyclosporine (CsA) is better than treatments with large-dose CsA for small bowel transplantation.

METHODS: Two-step segmental small bowel transplantation was performed in pigs and followed by treatment with either no, low-dose or high-dose CsA, which was followed by TW, a traditional Chinese medicine, or not.

RESULTS: The transplanted pigs receiving no CsA developed organ rejection, as did the pigs who received the low-dose CsA treatment alone; the mean survival time of the grafts was 12 ± 8.2 ± 7 d and 12 ± 4.2 ± 6 d respectively. Of the 4 transplanted pigs receiving the high-dose CsA for 100 d and then the TW treatment, 2 required euthanasia for severe pneumonia that developed on day 92 and 97 respectively, and the other 2 survived more than 348 and 327 d respectively. Of the 5 transplanted pigs receiving low-dose CsA for 100 d and then the TW treatment, all survived for 243 ± 2.90 ± 9 d and none succumbed to infection.

CONCLUSION: We are the first to use TW in small bowel transplantation and to show that TW can be a powerful and effective anti-rejection agent when applied in conjunction with the standard immunosuppressant CsA.

Key words: Tripterygium wolfordii intestine; Small/transplantation cyclosporine graft rejection

Li N, Li JS, Liao CX, Li YS. Anti-rejection therapy with tripterygium woifordii and low-dose cyclosporine for small bowel transplantation in pigs. *World J Gastroenterol* 1996; 2(1): 36-40 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i1/36.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i1.36

INTRODUCTION

The successful application of small bowel transplantation as a treatment for short gut syndrome is reliant on postoperative administration of the immunosuppressive agent cyclosporine A (CsA). While CsA is commonly used to prevent organ rejection and graft versus host disease (GVHD) in various transplantation cases, small bowel transplantation cases require much larger doses (15-20 mg/kg) to achieve the same results[1-6]. However, in general, allograft recipients should be maintained on the lowest possible doses of immunosuppressive agents in order to avoid development of lethal infections and lympho-proliferative disorders, as well as to minimize organ toxicity caused by the drug. In an overview of small bowel transplantation that was published in 1990, Wood[4]concluded that clinical intestinal transplantation would have to await further refinements in immunosuppression before it reaches its maturity as a successful treatment. We, however, have performed successful segmental small bowel transplantations in pigs by using low-dose CsA in combination with tripterygium wolfordii (TW), a traditional Chinese medicine, since Mar. 1992.

MATERIALS AND METHODS

Surgical technique

Two animals were prepared for small bowel transplantation involving one another. Each animal thus served as both graft donor and recipient. The two-step segmental small bowel transplantation was performed as described earlier[5]. Briefly, in the first-step operation (heterotopic transplantation), a segment of 5 m in length (approximately 40% of the entire intestine) was isolated. The mid portion of this segment (1 m in length) was reserved for use as a donor organ and the other 4 m of intestine was discarded. After the vascular bed and intes-

Table 1 Technical failures

Group	Animal No.	Survival days	Cause of death
I	1	5	Venous thrombosis
	3	1	Venous thrombosis
	5	0	Anesthetic
	9	1	Transplant intussusception
	10	32	Anesthetic, Rejection?
II	11	1	Venous thrombosis
	13	4	Transplant intussusception
	16	4	Arterial thrombosis
	17	7	Arterial thrombosis
III	19	1	Venous thrombosis
	22	0	Anesthetic
	26	0	Anesthetic
IV	29	3	Venous thrombosis
	31	4	Venous thrombosis
	33	6	Arterial thrombosis

Table 2 Immunosuppression and survival

Group	Immunosuppressant	Animal No.	Survival days	Cause of death
I	None	2	14	Rejection
		4	10	Rejection
		6	10	Rejection
		7	16	Rejection
		8	14	Rejection
II	High-dose CsA	12	92	Infection
		14	398	Survival
		15	97	Infection
		18	> 419	Survival
III	Tw + low-dose CsA	20	> 274	Survival
		21	> 356	Survival
		23	166	Ileus
		24	135	Unknown
		25	> 285	Survival
IV	Low-dose CsA	27	16	Rejection
		28	14	Rejection
		30	10	Rejection
		32	10	Rejection
		34	12	Rejection

TW: Tripterygium woilfordii.

tinal lumen were irrigated, the allograft artery and vein were anastomosed orthotopically to the recipient superior mesenteric artery and vein in an end-to-end fashion. Both ends of the graft were exteriorized with the stomas, and the native bowel continuity was restored. The second-step operation (orthotopic transplantation) was performed 4-6 wk after the heterotopic transplantation, if both the recipient and graft had survived. The heterotopic graft was freed from the enterostomies. Both ends of the graft were anastomosed with the recipient bowel in an end-to-end fashion.

Animal groups

Thirty-four outbred white pigs weighing 20 ± 1.5 kg were randomly assigned to one of four groups (below) for study. Genetic diversity in the population was assured by using donor recipient pairs from separate litters, except for Group Ⅰ in which the donor-recipient pairs came from the same litters.

Group Ⅰ (n = 10): control group, received no immunosuppressant.

Group Ⅱ (n = 8): CsA group, received CsA at 15 mg/kg intramuscularly provided on the day before the first-step operation and continuing for 60 d, with dosage being reduced to 10 mg/kg at 1 wk until day 60 post-transplantation and then again to 7.5 mg/kg until day 100 post-transplantation.

Group Ⅲ (n = 8): TW + low-dose CsA group, received TW at 2 mg/kg orally for 3 d prior to the first-step operation and for lifelong use post- transplantation, and also received CsA at 7.5 mg/kg intramuscularly for 7 d post-transplantation, with dosage being reduced to 5 mg/kg from day 8 to day 60 post-transplantation and again to 2.5 mg/kg until day 100 post-transplantation.

Group Ⅳ (n = 8): low-dose CsA group, received CsA alone (without TW) *via* administration as described for Group Ⅲ.

Group Ⅴ (n = 7): TW group, consisting of animals that survived more than 100 d from Groups Ⅱ and Ⅲ who then received only TW at 2 mg/kg orally.

Postoperative monitoring

Animals were evaluated daily postoperatively. Clinical characteristics and appearance of the exteriorized stomas of the transplanted intestines were recorded. If the graft mucosal biopsies were found to contain necrotic tissue, the animals were sacrificed. All recipient animals were autopsied upon death.

Serum protein, aspartate aminotransferase, alanine aminotransferase, total bilirubin, blood urea nitrogen and serum creatinine were measured on postoperative day 40 and 80.

Whole blood CsA levels were measured on postoperative day 5, 10, 20, 40, 60, 80 and 100, on a sampling schedule just prior to administration of daily CsA. The radioimmunoassay kit for CsA measurement was purchased from Abbott Inc. (United States).

While the grafts were in a heterotopical position, mucosal biopsies of the transplant were obtained at 2-d intervals, with a suction biopsy instrument. The specimens were fixed in 10% phosphate-buffered formalin, embedded in paraffin, and serially sectioned (6 microns) for analysis by staining with hematoxylin and eosin and light microscope.

Statistical analysis

All data are expressed as the mean ± SD. Survival times were compared using rank sum analysis, and for other parameters the Student's test and variance analysis was used.

RESULTS

Of the total 34 transplants that were conducted, 15 (44.1%) of

Table 3 Mean survival period with or without percutaneous transhepatic cholangial drainage prior to operation

Group, *n*	Postoperative days							
	5	10	20	40	50	80	60	100
Group Ⅱ, 4	1476 ± 11.6[b]	902 ± 79[b]	894 ± 89[b]	896 ± 86[b]	878 ± 87[b]	495 ± 109[b]		-425, 388
Group Ⅲ, 5	429 ± 79	304 ± 33	298 ± 34	301 ± 39	289 ± 36	157 ± 30	155 ± 35	
Group Ⅳ, 5	446 ± 63	304 ± 48						

[b]$P < 0.01$ *vs* group Ⅲ and Ⅳ.

Table 4 Results of liver kidney functions in Groups Ⅱ and Ⅲ

Biochemistry	Preoperative	POD 40		POD 80	
	Control	Group Ⅱ	Group Ⅲ	Group Ⅱ	Group Ⅲ
ALT (nkat)	280 ± 40	429 ± 89[a]	350 ± 53	629 ± 257[a]	327 ± 87
AST (nkat)	410 ± 90	771 ± 119[a]	690 ± 132	1267 ± 444[a]	410 ± 114
LDH (U/L)	301 ± 72	445 ± 69	370 ± 79	567 ± 147[a]	333 ± 140
AKP (nkat)	840 ± 184	942 ± 319[a]	837 ± 177	1304 ± 593	750 ± 196
Bil (μmol/L)	11.3 ± 5.5	17.1 ± 7.4	16.1 ± 5.7	31.2 ± 23.7	14.7 ± 7.4
BUN (mmol/L)	3.2 ± 0.8	3.2 ± 0.4	3.7 ± 1.1	4.3 ± 0.7	3.4 ± 0.8
Cr (μmol/L)	89 ± 26	28 ± 34	95 ± 27	93 ± 27	83 ± 20

[a]$P < 0.05$ *vs* preoperative and group Ⅲ. POD: Postoperative days.

Table 5 Results of serum biochemistry in group Ⅳ

Biochemistry	Preoperative	Postoperative days		
	(Control)	130	160	190
Sodium (mmol/L)	153 ± 8	150 ± 8	153 ± 10	153 ± 9
Potassium (mmol/L)	5.7 ± 1.0	5.9 ± 0.9	5.9 ± 1.0	5.8 ± 1.1
Chloride (mmol/L)	101 ± 5	100 ± 4	101 ± 5	102 ± 3
Total protein (g/L)	87.8 ± 6.8	85.3 ± 8.1	84.3 ± 4.1	85.0 ± 5.1
Albumin (g/L)	37.2 ± 5.9	36.0 ± 3.3	37.0 ± 2.8	37.0 ± 2.2
Globulin (g/L)	50.6 ± 4.4	49.3 ± 5.5	47.3 ± 2.2	48.0 ± 5.4
A/G	0.74 ± 0.14	0.74 ± 0.06	0.78 ± 0.06	0.78 ± 1.1
ALT (nkat)	280 ± 40	304 ± 37	283 ± 101	300 ± 53
AST (nkat)	410 ± 90	496 ± 184	450 ± 175	484 ± 168
LDH (u/L)	301 ± 72	289 ± 65	273 ± 66	278 ± 74
AKP (nkat)	840 ± 184	859 ± 97	805 ± 104	824 ± 127
Bilirubin (μmol/L)	11.3 ± 5.5	13.5 ± 5.3	12.4 ± 5.1	12.6 ± 4.3
BUN (mmol/L)	3.2 ± 0.8	3.5 ± 0.8	3.4 ± 0.9	3.5 ± 0.9
Cr (μmol/L)	89 ± 26	88 ± 17	86 ± 22	92 ± 28

Survival > 200 d ($n = 5$).

the animals died of technical failures, including anesthetic accidents, (Table 1) within the first 7 d post-transplantation; all these animals were excluded from analyses. If the 4 animals that died as a result of anesthetic accident were excluded, the rate of technical failure was 36.7% (11/30).

Immunosuppression and survival

The immunosuppressive regimens used and corresponding survival figures are presented in Table 2. The animals in Group Ⅰ ($n = 5$) that received no immunosuppression, as well as those in Group Ⅳ ($n = 5$) that received low-dose CsA, died of rejection on post-transplantation days 12.8 ± 2.7 and 12.4 ± 2.6, respectively. At autopsy, it was apparent that the transplanted bowel had deteriorated into a hard, whitish, irregular mass. Histological analysis showed typical signs of rejection that were specific to the transplanted organ, and no obvious changes were observed in the liver, spleen or lymph nodes of the recipients.

The animals in Group Ⅱ ($n = 4$) that received high-dose CsA survived for 251.5 ± 181.5 d post-transplantation. In this group, 2 animals were sacrificed on post-transplantation days 92 and 97, respectively, due to severe pulmonary infection; the remaining 2 pigs survived to post-transplant day 398 and 419, respectively. The animals in Group Ⅲ ($n = 5$) that received TW + low-dose CsA survived 243.2 ± 90.9 d ($P < 0.05$ compared to Group Ⅳ). In this group, 3 animals remained alive until post-transplantation day 274, 285 and 356, respectively, 1 animal died suddenly at post-operative day 135 of no identifiable cause (despite autopsy evaluation), and 1 animal succumbed to obstruction of the native intestine on post-transplantation day 166. Although 2 pigs in Group Ⅱ and 2 in Group Ⅲ died between post-transplantation day 92 and 166, there was no evidence of acute or chronic rejection at autopsy.

Pathology

The histological structure of the transplanted intestines was normal for the animals in both Group Ⅱ and Group Ⅲ, comparable to that of the native intestine. The specimens from Group Ⅰ and Group Ⅳ showed signs of rejection, including decreased mucosal depth accompanied by atrophic villi, reduced glandular structures and degeneration of arteriole in the laminal propria with infiltration of mononuclear cells. As rejection progressed, patch erosion of the mucosal surface occurred along with loss of epithelial cells. Finally, the mucosal structure disappeared entirely and mucosal biopsy specimens consisted solely of necrotic tissue. The earliest pathological indication of rejection was seen at 7.6 ± 1.7 d after transplantation. Mucosal necrosis of the transplanted intestine emerged at 12.4 ± 2.6 d after surgery. Results of whole blood CsA and serum biochemistries are listed in Tables 3-5.

DISCUSSION

Prognosis and resectability rate

Rejection is the main challenge to success of intestinal transplantation. In the 1960s and 1970s, azathioprine was the major drug used to combat rejection of the donated organ; yet, during this period there were almost no reports of successful intestinal transplantation. In the 1980s, CsA became the most popular and effective anti-rejection agent in use, and the survival rate of small bowel graft was greatly prolonged[6]. Although reports of successful small bowel transplantation were present in the literature after 1988[7], the long-term survival rate remained low—less than 36% of the animals survived for 100 d[3]. Here, we demonstrate for the first time that

TW is a powerful anti-rejection agent, as evidenced by all 5 of the pigs in our study who received TW along with low-dose CsA surviving for more than 100 d.

Although CsA has been widely accepted as an effective immunosuppressive agent for small bowel transplantation, large doses have been required to prevent rejection and GVHD. Grant[2] applied a dosage of 8 mg/kg CsA and 1 mg/kg prednisolone for pigs receiving total intestinal transplantation, but all the pigs in that study died of acute rejection within 15 d. According to Ricour[8] and Revillon[9], 14 pigs with blood CsA level < 750 μg/L (equal to a CsA plasma concentration of 250 mg/L) died of rejection within 8-75 d after the operation. Pritchard's experiment[10] revealed that 4 of the pigs with blood CsA concentration of 200 μg/L died within 15 d after operation because of acute rejection, with the other 6 animals with blood CsA level of 380 μg/L dying within 29 ± 18 d and still another 8 pigs with CsA concentration reaching 610 μg/L dying within 34 ± 33 d. This evidence indicated that CsA itself was unable to inhibit rejection if blood level was < 750 μg/L.

We obtained similar results as above, with 4 pigs in Group Ⅱ surviving without rejection when the CsA blood level was maintained > 800 μg/L for 60 d; acute rejection manifested in the 5 pigs that received half the dosage of CsA (blood level 300-500 μg/L). Thus, we concluded that the minimal effective concentration of CsA was -750 μg/L, which is equivalent to a daily injection of 10 mg/kg of CsA. Although large dosage of CsA can inhibit rejection of the transplanted small bowel, few animals in our study survived for 100 d and most succumbed to severe infection. In Grant's study[2], a daily intravenous administration of CsA at 20 mg/kg was provided for intestinal transplanted pigs followed by oral ingestion of CsA (25 mg/kg) 3 wk later. Unfortunately, all of the animals in that study died of infection within 28 d. In another study, Kaneko[11] gave intravenous injection of CsA (15 mg/kg) to intestinal transplanted pigs for 30 d followed by oral CsA at 30 mg/kg; as a result, 69% (9/13) of these animals died within 38 ± 14 d due to infection. Similarly, Kimura[3] reported that among 11 recipient pigs of segmental jejunal transplantation receiving 10 mg/kg of CsA intramuscular injection, 7 animals (63.6%) died of infection, 1 died of rejection, and only 4 pigs survived for 100 d. Pritchard[10] reported that pigs of segmental jejunal transplantation were given 25 mg/kg of CsA orally and 40% of the animals died of infection, with only 2/47 surviving for 100 d. In our experiment, 4 pigs in our group Ⅱ suffered from lung infection at 35-50 d after the operation and 2 of those animals died. We, thus, concluded that large-dose CsA could not only inhibit rejection but also increase risk of severe infection.

Large-dose CsA can cause liver and kidney damage. The nephrotoxicity of CsA is widely acknowledged. Clinical data have revealed that CsA causes severe kidney damage if its concentration is maintained > 800 ng/mL for a long period. In our study, instances of kidney damage were not prominent; this finding may reflect species variation. However, animals in group Ⅱ showed a certain degree of liver damage; specifically, blood ALT, AST and LDH on post-transplantation days 40 and 80 were significantly higher than those of other groups; autopsy of the 2 animals that died in group Ⅱ showed degeneration and necrosis of liver cells in the lobules. Thus, we concluded that large dosage of CsA was not the ideal regimen to avoid rejection.

TW, a traditional Chinese medicine, is an anti-inflammatory, antibacterial, anti-fertilization and immunosuppressive agent. It is already in wide use for the treatment of autoimmune diseases, but has not yet been generally extended towards the prevention of rejection after major organ transplantation. We are the first to use TW to treat small bowel transplantation and find that TW is an effective anti-rejection agent. Ultimately, our results suggest that TW can partly replace CsA in the prevention and treatment of acute rejection in the early stage after small bowel transplantation and, in the later period, can be used alone to protect against chronic rejection.

Combined drug therapy can help to protect against rejection, thereby reducing related side effects and achieving better outcome. In the field of intestinal transplantation, the effective blood concentration of CsA can cause not only liver and kidney damage, but also severe infection[13]. In our study, combined drug therapy was adopted in group Ⅲ and the dose of CsA was only half that administered to the animals in group Ⅱ. Although rejections were effectively controlled in both groups, all of the 4 pigs in group Ⅱ manifested infection within 35-50 d after the transplantation. Both liver function test and biopsy revealed liver cell damage, and only 2 of the animals survived for 100 d. All of the 5 pigs in group Ⅱ survived more than 100 d, and 2 of them manifested transient mild lung infection but did not show any signs of liver damage. Thus, combination treatment of TW with low-dose CsA appeared to be an effective and reasonable regimen against rejection. It could produce the same effect as that of large dosage of CsA without causing significant infection and liver damage, supporting a high rate of long time survival.

TW is a toxic herb and its toxicity is dose-related. The symptoms of chronic toxicity in experimental animals include hair shedding, weight loss, white blood cell count reduction and damage to the heart, liver and kidney. In our study, 7 pigs in group Ⅳ took TW for as long as 135-419 d without developing any of these symptoms.

We postulated that the mechanism underlying the immunosuppressive effect of TW involves inhibition of cytokines. Our primary study has shown differences in IL-2 and IL-6 levels in animals of groups Ⅱ and Ⅲ, suggesting that TW may act *via* inhibition of IL-2 and IL-6 production; further investigation is warranted.

The regime of TW + low-dose CsA will be very cost effective. It is commonly known that CsA is very expensive, but TW is much cheaper. The daily cost of TW per kg body weight is only 1/10 of that of CsA and thus is promising for clinical care.

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S- Editor: Filipodia **L- Editor:** Jennifer **E- Editor:** Liu WX

Published by **Baishideng Publishing Group Inc**
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ISSN 1007-9327