1749

Effect of Anticomplement Agent K-76 COOH in Hamster-to-Rat and Guinea Pig-to-Rat Xenotransplantation

M. Tanaka, N. Murase, H. Miyazawa, Q. Ye, R. Manez, S. Todo, A.J. Demetris, M. Nomoto, W. Miyazaki, and T.E. Starzl

THE anticomplement agent K-76 COOH (K76), which mainly inhibits C5 activation, has been effectively used to mitigate experimental immune complex glomerulonephritis and ulcerative colitis.[1,2] The effectiveness of K76, alone or in combination with FK 506 and cyclophosphamide (CP), in preventing xenograft rejection was studied using the hamster-to-rat and guinea pig-to-rat heart transplantation model.

MATERIALS AND METHODS

Inbred male Lewis rats weighing 200 to 250 g, outbred male Syrian hamsters weighing 100 to 150 g, and outbred Hartley guinea pigs weighing 250 to 300 g were obtained from Charles River Laboratories (Wilmington, Mass). Heterotopic abdominal heart transplantation was performed using the technique described by Ono and Lindsey.[3] Rejection was diagnosed by the cessation of heart beat by palpation, followed by direct inspection.

K76 (donated by Otsuka Pharmaceutical Co. Ltd, Tokushima, Japan), suspended in normal saline and adjusted to pH 7.8, intramuscular FK 506 (Fujisawa Pharmaceutical Co, Osaka, Japan), and oral CP were used in this study. Treatment schedules for xenograft recipients are listed in the Table 1. In hamster-to-rat transplantation, K76 (200 mg/kg) was intravenously injected 30 minutes before graft revascularization and administered every 12 hours for the first 6 days after grafting. Two mg/kg/d of FK 506 was injected intramuscularly on days 0 to 5, 1.0 mg/kg/d on days 6 to 30, and 0.5 mg/kg/d every other day thereafter. CP was given at a daily dose of 5.0 mg/kg on days −1 to 7. In the guinea pig-to-rat transplantation, various doses of K76 (100 to 400 mg/kg) were given 30 minutes before revascularization as a single bolus injection. An additional group of animals were pretreated with FK 506 (1.0 mg/kg/d) and CP (7.5 mg/kg/d) for 10 days (day −10 to −1).

The Mann-Whitney U test was used for statistical analysis of xenograft survival and $P < .01$ was considered significant.

The development of anti-hamster antibodies in rat recipients was studied by complement-dependent lymphocytotoxic antibody assay using serially diluted serum samples (heat inactivated, 56°C, for 30 minutes), and hamster lymphocytes as described previously.[4] Baby rabbit serum (Cedarlane Laboratories Ltd) was used as a source of complement. The cytotoxic antibody titer was defined as the highest serum dilution with more than 51% of cell lysis.

From the Pittsburgh Transplant Institute, Department of Surgery and Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania (M.T., N.M., H.M., Q.Y., R.M., S.T., A.J.D., M.N., T.E.S.), and All Children's Hospital and Department of Pediatrics, University of South Florida, St. Petersburg, Florida (W.M.).

Address reprint requests to Mitsuko Tanaka, MD, 4C Falk Clinic, 3601 Fifth Ave, Pittsburgh, PA 15213.

0041-1345/95/$3.00/+0

Table 1. Effect of K76 COOH in Hamster-to-Rat and Guinea Pig-to-Rat Heart Transplantation

		Treatment					
	Group	K76 (mg/kg/d)	FK 506 (mg/kg/d)	CP (mg/kg/d)	n	Median[d] Survival	P
Hamster to Rat	1	–	–	–	6	3.0	
	2	400[a]	–	–	6	4.0	<.005*
	3	–	2.0[b]	–	6	4.0	<.005*
	4	–	–	5.0 (d −1–7)	3	4.0	NS*
	5	–	2.0[b]	5.0 (d −1–7)	9	5.0	<.002*
	6	400[a]	2.0[b]	–	4	4.5	=.01*
	7	400[a]	–	5.0 (d −1–7)	4	7.0	=.01*
	8	400[a]	2.0[b]	5.0 (d −1–7)	7	61.0	<.005*
Guinea Pig to Rat	9	–	–	–	10	0.17	
	10	100 × 1	–	–	5	1.70	<.005**
	11	200 × 1	–	–	10	10.15	<.0005**
	12	300 × 1	–	–	5	4.00	<.005**
	13	400 × 1	–	–	3	5.90	=.01**
	14	200 × 2	–	–	6	18.09	<.005**
	15	200[c]	1.0 (d −10 to −1)	7.5 (d −10 to −1)	5	24.00	<.005**

[a]K76 (200 mg/kg) was intravenously injected 30 minutes before graft revascularization and continued every 12 hours for 6 days.
[b]FK 506 was intramuscularly injected at doses of 2.0 mg/kg/d (d 0–5), 1.0 mg/kg/d (d 6–30), and 0.5 mg/kg/d (every other day, d 31–100).
[c]K76 was given 30 minutes before revascularization and every 6 to 12 hours after Tx.
[d]Median survival of groups 1 to 8 was expressed in days, and groups 9 to 15 in hours.
*vs group 1.
**vs group 9.

RESULTS

Hamster-to-Rat Transplantation (Table 1, Groups 1 to 8)

Untreated heart grafts were rejected in 3 days. When K76, FK 506, or CP was used as a single therapy (groups 2 to 4) or when two of three drugs were combined (groups 5 to 7), graft survival was slightly prolonged to 4 to 7 days. In animals treated with K76, FK 506, and CP, graft survival was dramatically enhanced, with a median of 61 days (group 8). In these animals, anti-hamster lymphocytotoxic antibody titers rapidly increased during the first 5 days, from a normal of 2^5 to a high of 2^8. Subsequently titers returned to 2^6 and remained low for more than 28 days.

Guinea Pig-to-Rat Transplantation (Table 1, Groups 9 to 15)

Guinea pig hearts were hyperacutely rejected within 0.27 hours by untreated rats. K76 doses of 100 and 200 mg/kg resulted in prolonged heart graft survival of 1.70 and 10.15 hours, respectively. However, higher doses of K76 caused animal death with a beating graft in 6 of 8 recipients (groups 12 and 13). Repeated doses of K76 further prolonged graft survival to 18.09 hours, with 83% of animal deaths with beating grafts (group 14). The addition of FK 506 and CP pretreatment to K76 resulted in further prolongation of survival to 24 hours (group 15).

DISCUSSION

In the hamster-to-rat model, rejection is mainly caused by rapidly increasing xenospecific antibodies and subsequent complement activation, whereas in the guinea pig-to-rat model, the rejection was believed to cause direct complement activation via the alternative pathway and result in hyperacute rejection. As shown in this study, K76 was effective in prolonging the hamster and guinea pig xenograft survival in rats by inhibiting both types of complement activation. A pharmacokinetic study of K76 revealed that an intravenous dose of 200 mg/kg effectively depletes/suppresses complement activity in normal rats. ACH50 and C3 level were reduced to less than 50% of the initial value for 6 hours, whereas the inhibition of CH50 was less dramatic and only 20% suppression was seen for 3.5 hours. When K76 was injected every 12 hours for the first 6 days, there was little prolongation of hamster graft survival. The addition of FK 506 and low-dose CP to this treatment protected the hamster graft from a vigorous antibody reaction and led to prolonged survival. However, the results obtained with K76, FK506, and CP are inferior to those of previous studies, in which suppression of B-cell proliferation and inhibition of antibody production were obtained by higher doses of CP and FK 506.

On the other hand, a single bolus intravenous injection of K76 induced prolonged guinea pig heart graft survival, which could not be achieved by FK 506 and CP. This remarkable effect of K76 is better than the previous reports using intraperitoneal K76 administration.

These results suggest that complement inhibition using K76 is effective for xenotransplantation that is mainly mediated by direct complement activation, such as the guinea pig to rat. When xenograft rejection is mediated by xenospecific antibodies and classical pathway complement activation, as in the hamster-to-rat model, inhibition of antibody production may be more effective than K76 in preventing rejection.

REFERENCES

1. Iida H, Izumino K, Asaka M, et al: Clin Exp Immunol 67:130, 1987

2. Kitano A, Matsumoto T, Nakamura S, et al: Dis Colon Rectum 35:560, 1992

3. Ono K, Lindsey ES: J Thorac Cardiovasc Surg 57:225, 1969

4. Murase N, Starzl TE, Demetris AJ, et al: Transplantation 55:701, 1993