Therapeutic Synergism Between Low-Dose FK 506 and Antimetabolites in Rat Allogeneic Heart Transplantation

1748

M. Tanabe, S. Todo, N. Murase, W. Irish, H. Miyazawa, S. Fujisaki, and T.E. Starzl

FK 506 HAS HAD A major beneficial effect on the survival of transplanted organs. However, the use of FK 506 is limited by its toxicity. To circumvent this problem, we attempted to amplify the therapeutic efficacy of low-dose FK 506 by the combined use of various antimetabolites, including RS-61443 (RS),[1] mizoribine (MIZ),[2] and azathioprine (AZA). Therapeutic interaction was evaluated with rat allogeneic heart transplantation.

MATERIALS AND METHODS

Animals and Operation

Inbred male Lewis (LEW, *RT1*^{l}) and ACI rats (*RT1*^{a}) (Harlan Sprague-Dawley Inc, Indianapolis, Ind) served as recipients and donors, respectively. Cardiac allografting was performed by the method of Ono and Lindsay,[3] and rejection was established by cessation of heart beat followed by histologic examination.

Immunosuppression

All immunosuppressive regimens were continued for 14 days, starting on the day of heart transplantation. According to our previous report,[4] a daily dose of 0.04 mg/kg was selected as a minimally effective dose of FK 506 between no effect and clinical response. Serial oral doses of each antimetabolic drug from subclinical to lethal (shown in Table 1) were examined both as a monotherapy or in combination with 0.04 mg/kg FK 506. RS-61443 (Syntex Inc, Palo Alto, Calif) was used in a special vehicle (0.5% carboxymethylcellulose, 0.4% polysorbate 80, 0.9% benzyl alcohol, and 0.9% sodium chloride in distilled water). Mizoribine (Bredinin, Asahi Chemical Industry Co Ltd., Tokyo, Japan) and azathioprine were suspended in distilled water.

Table 1. The Effect of Antimetabolite Treatment With or Without the Addition of FK 506

Antimetabolites (mg/kg/d)	Median Graft Survival Days (n) Without FK 506[a]		With FK 506[a]		CI	
	—	9	(11)	6	(7)	—
MIZ	2.5	7	(5)	21*	(7)	0.64
	5	9	(6)	19*	(6)	0.86
	10	12	(6)	26*	(5)	0.70
	15	15	(5)	21	(5)	—
	20	14	(5)	9	(5)	—
	30	8	(5)	4	(5)	—
AZA	5	6	(5)	11	(6)	2.27
	30	11	(5)	28*	(5)	0.75
	45	19	(5)	32*	(5)	0.80
	60	11	(5)	30	(5)	—
	90	5	(4)	6	(5)	—
RS	20	13	(7)	22*	(6)	1.00
	40	23	(6)	27	(5)	1.16
	60	19	(6)	13	(5)	—
	80	4	(4)	4	(4)	—

*Survival significantly greater ($P < .05$) than that with antimetabolite monotherapy and fixed low-dose FK 506 monotherapy.
[a]Intramuscular FK 506 (0.04 mg/kg/d) for days 0 to 13.

Statistical Analysis

The Mann-Whitney *U* test and/or Kruskall-Wallis test was used to analyse the survival difference between the treatment groups. The results were considered significant if $P < .05$. The interaction between FK 506 and these antimetabolites was assessed by the combination index (CI), which was calculated by the median effect analysis of Chou and Kahan.[5,6] CI values less than 1.0 suggest synergism; above 1.0 antagonism; and equal to 1.0 additivity.

RESULTS

With antimetabolite monotherapy, graft survival was prolonged with dose increments until this improvement was interrupted at higher doses by the mortality of overdosage. The peak median graft survival was 15 days for MIZ (15 mg/kg/d), 19 days for AZA (45 mg/kg/d), and 23 days for RS (40 mg/kg/d). The difference was significant between RS and MIZ ($P = .04$) but not between RS and AZA. Indefinite graft survival (>100 days) in two experiments was achieved only with 60 mg/kg/d RS.

All antimetabolites when added to low-dose FK 506 provided better results at some doses ($P < .05$) than when low-dose FK 506 or antimetabolite was given alone. This was accomplished at doses of 2.5, 5.0, and 10.0 mg/kg/d MIZ, 30 and 45 mg/kg/d AZA, and 20 mg/kg/d RS. Among these combined therapy groups, the peak median survival of 32 days using 45 mg/kg/d AZA was significantly longer than that of MIZ (26 days at 10 mg/kg/d MIZ, $P = .03$) or RS (27 days at 40 mg/kg/d RS, $P = .43$).

The CI between each dose of antimetabolite and FK 506 was calculated from forgoing data excluding toxic doses of antimetabolite (no higher than the doses with peak median survival). These were 0.64 to 0.86 for MIZ, 0.75 to 2.27 for AZA, and 1.00 to 1.16 for RS.

From The Pittsburgh Transplant Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania.

Supported by a project grant DK29961 from the National Institutes of Health, Bethesda, Maryland, and a research grant from the Uehara Memorial Foundation, Tokyo, Japan.

Address reprint requests to Thomas E. Starzl, The Pittsburgh Transplant Institute, 3601 Fifth Avenue, 5C Falk Clinic, Pittsburgh, PA 15213.

0041-1345/95/$3.00/+0

DISCUSSION

A benefit of combined immunosuppressive therapy with low-dose FK 506 and antimetabolites for heart transplantation from ACI to LEW rats was clearly shown in this study. Therapeutic synergism was present when FK 506 was combined with either MIZ (2.5, 5.0, and 10 mg/kg/d) or AZA (30, 45 mg/kg/d), whereas RS interacted with FK 506 no more than additively. It is worth noting that therapeutic synergism with low-dose FK 506 was particularly pronounced at the subclinical dose of MIZ (2.5 mg/kg/d). Of the three antimetabolites, RS gave the best graft survival when used alone, but the best overall result was obtained by combining AZA and FK 506.

A conventional explanation for drug synergism is the different site of action of the combined agents. Suppression of allospecific T-cell immunity by FK 506 is explained by inhibition of the production of IL-2 and its receptor expression.[7] In contrast, MIZ, AZA, and RS inhibit purine biosynthesis, curtailing the DNA synthesis necessary for cell division, including that of activated T and B lymphocytes.[1,2,8] Synergism resulting from these diverse antilymphocytic mechanisms has been observed in vitro,[9] and its clinical utility was supported by the in vivo results of this study.

REFERENCES

1. Nelson PH, Eugui E, Wang CC, et al: J Med Chem 33:833, 1990
2. Mizuno K, Tsujino M, Takada M, et al: J Antibiot (Tokyo) 27:775, 1974
3. Ono K, Lindsay ES: J Thorac Cardiovasc Surg 7:225, 1969
4. Murase N, Todo S, Lee P-H, et al: Transplant Proc 19:71, 1987
5. Chou TC: J Theor Biol 39:253, 1976
6. Kahan BD, Gibbons S, Tejpal N, et al: Transplantation 51:232, 1991
7. Tocci MJ, Matkovich DA, Collier KA, et al: J Immunol 143:718, 1989
8. Elion GB: Science 244:41, 1989
9. Zeevi A, Duquesnoy R, Eiras G, et al: Transplant Proc 20:220, 1988