行政院國家科學委員會補助專題研究計畫成果報告

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※ 心臟移植後急性排斥血中蛋白質的變化 ※

※ Plasma protein changes ※

※ in acute cardiac allograft rejection ※

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計畫類別：個別型計畫

計畫編號：NSC 89－2314－B－002－286－

執行期間：89 **年** 8 **月** 1 **日至** 90 **年** 7 **月** 31 **日**

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一、摘要

Rejection is the major cause of morbidity and mortality during the first year following transplantation. We have reported that 63% of our heart recipients had rejection within one year and the mean number episodes in the first year after transplant was 1.14. Diagnosis and treatment of acute rejection have evolved tremendously since the first human heart transplantation in the 1960s. At present the detection of rejection relies mostly on the pathological examination of endomyocardial biopsy specimens. The majority of cardiac transplant centers have a fixed schedule for patients to undergo endomyocardial biopsy. However, this approach is invasive and is associated with significant morbidity and mortality. The easiest way for early detection of acute cellular rejection is to find the rejection-associated molecules in the blood of the recipients.

These serum markers is supposed to be released into the cardiac veins and finally drained into coronary sinus and right atrium. We report our preliminary results of several serum markers in coronary sinus and peripheral blood in patients with heart transplantation.

From the standpoint of immune response in cardiac allograft rejection, the possible candidates of rejection-associated molecules included:

1. Conventional markers: Troponin-I.
2. Acute phase proteins: C-reactive protein (CRP).
3. Cytokines: interleukin-2 (IL-2), tumor necrosis factors-alpha (TNF).
4. Adhesion molecules: ICAM-1, P-selectin.

There were 71 blood samples from 51 patients of cardiac post-transplantation. The post-transplantation period ranged from one week to five years. For the samples from the same subjects, the minimal sampling interval was 12 days and the maximal was two years. Each sample was considered as independent, because within-subject samples were taken with adequate intervals between them.

Same special markers between coronary sinus (CS) and peripheral blood (PB) were significantly correlated. Among them, there were also significant correlations. P-selectin may correlate with renal function. ICAM may correlate with liver function. CRP may correlate with WBC. Lots of variables differed between early and late HTx groups and between infection and non-infection groups. TNF-PB differed between CAD and non-CAD groups (p=0.0498). IL2-PB differed between rejection and non-rejection groups (p=0.0409). CRP-PB differed between long and short survivors (p=0.0273). Troponin-I-PB differed between statin users and non-users (p=0.0177). CHO differed between Tapal users and non-users (p=0.0270).

Further understanding of those markers was needed for more interpretation.

二、緣由與目的

Background and Significance

Rejection is the major cause of morbidity and mortality during the first year following transplantation [1]. We have reported that 63% of our heart recipients had rejection within one year and the mean number episodes in the first year after transplant was 1.14 [2]. Most of the rejection episodes occurred during the first year after transplantation. Diagnosis and treatment of acute rejection have evolved tremendously since the first human heart transplantation in the 1960s. At present the detection of rejection relies mostly on the pathological examination of endomyocardial biopsy specimens. The majority of cardiac transplant centers have a fixed schedule for patients to undergo endomyocardial biopsy [2]. However, this approach is invasive and is associated with significant morbidity and mortality.

More recently, heart transplant centers have investigated the uses of noninvasive methods to diagnose acute allograft rejection, such as enzymatic markers, electrocardiography, nuclear medicine, and echocardiography, with limited success.

At this time endomyocardial biopsy is still the most clinically applicable technique for the detection of acute cellular rejection. However, endomyocardial biopsies are impractical to use as a screening tool because of the cost, discomfort to the patient, and, although infrequent, potential serious complications. The easiest way for early detection of acute cellular rejection is to find the rejection-associated molecules in the blood of the recipients.

Cardiac enzymes [3-5], cytokines [6-9], inflammatory mediators [10] and adhesion molecules [11] have been studied in patients with heart transplantation. Although these parameters are elevated often in the presence of rejection, their specificity and sensitivity do not allow them

to replace routine performance of biopsy for monitoring rejection after transplantation. However, they do have prognostic significance.

In summary, that a lack of sensitivity of these potential serum markers may be relates to the fact that rejection of an allograft produces low-grade and focal injury resulting in a small enzymatic leak, which was further diluted by the systemic circulation. Therefore, these markers in peripheral blood are unreliable and should not be used as a screening test for acute cellular rejection [12].

These serum markers is supposed to be released into the cardiac veins and finally drained into coronary sinus and right atrium. It makes a concentration gradient of serum markers between coronary sinus and systemic blood. The concept of “myocardial outflow” will help us to detect the abundant rejection-associated proteins in coronary sinus.

研究方法

Study patients:

From October 1987 to July 2000, we had performed 140 cases of heart transplantation. All patients admitted for endomyocardial biopsy are included in this study.

Protocol of immunosuppression: All patients received triple-drug immunosuppressive therapy according to our heart transplantation protocol [2].

Endomyocardial biopsy and grading of allograft rejection:

Endomyocardial biopsy was performed weekly in the first month, biweekly in the second month, monthly in the 6 months and yearly six months after transplantation. Rejection was defined as a clinical event leading to specific immunosuppressive intervention. The scale of the International Society for Heart Transplantation is used for grading of rejection. This scale ranges from grade 0, which denotes no sign of acute rejection through 1A, 1B, 2, 3A, 3B to grade 4 which denotes diffuse aggressive inflammation, myocyte necrosis and damage, edema, hemorrhage, and vasculitis [13]. Humoral rejection was diagnosed by the immunofluorescent staining of immunoglobulins and complement.

Chronic rejection: transplant coronary artery disease

For those patients surviving for more than six months after transplantation, coronary angiography was performed annually for surveillance of transplant CAD. Side-by-side comparisons were done. The diagnosis of transplant CAD was made from the evidence of any coronary artery irregularity or diffuse narrowing in proximal and distal vessels [2].

Clinical data collection

Cardiac events including myocardial infarction, left ventricular dysfunction, arrhythmia and sudden death were recorded. The recipient characteristics (age, sex, body weight, smoking, hypertension, diabetes, cholesterol level, triglyceride level and viral infection), donor characteristics (age, sex, preexisting coronary artery disease and allograft ischemic time), rejection episodes, medications and HLA match were recorded. Cytomegalovirus (CMV) infection was defined as a 4-fold rise in antibody, CMV inclusion body, or positive culture. Rejection was defined as a clinical event leading to specific immunosuppressive intervention.

Sample collection:

Under the guidance of fluoroscopy, we cannulate the coronary sinus through right internal jugular approach. The correct position in coronary sinus is verified by analysis of the blood oxygen saturation. Study blood samples are collected directly from coronary sinus. The samples are collected in vacuum tubes, kept in ice slush, and centrifugated. Plasma samples are then immediately frozen and stored until analysis. Control blood samples are collected from the peripheral blood. Blood of patients with acute rejection are collected serially before and after augmented immunosuppression.

Plasma determination:

From the standpoint of immune response in cardiac allograft rejection, the possible candidates of rejection-associated molecules included:

1. Conventional markers: Troponin-I
2. Acute phase proteins: C-reactive protein (CRP)
3. Cytokines: interleukin-2 (IL-2), tumor necrosis factors-alpha (TNF).
4. Adhesion molecules: ICAM-1, P-selectin

三、結果與討論

There were 71 blood samples from 51 patients of cardiac post-transplantation. The post-transplantation period ranged from one week to five years. For the samples from the same subjects, the minimal sampling interval was 12 days and the maximal was two years. Each sample was considered as independent, because within-subject samples were taken with adequate intervals between them. Non-parametric methods were chosen for all statistical analysis. Whitney-Mann test and Spearman correlation were used. Stata 6.0 was the statistical software utilized in the study (http://www.stata.com).

1.Same special markers between coronary sinus (CS) and peripheral blood (PB) were

significantly correlated. Among them, there were also significant correlations.

Spearman (non-parametric) Correlation (* denotes significant correlation)

	Psele1	Icam1	Tnf1	Il1	Crp1	Troi1	Psele2	Icam2	Tnf2	Il2	Crp2	Troi2
Psele1		*					*					
Icam1	*			*	*		*	*		*	*	*
Tnf1				*					*	*		
Il1		*	*						*	*		
Crp1		*				*	*	*			*	*
Troi1					*		*	*			*	*
Psele2	*	*			*	*					*	*
Icam2		*			*	*					*	*
Tnf2			*	*						*		
Il2		*	*	*					*			
Crp2		*			*	*	*	*				*
Troi2		*			*	*	*	*			*	

2.P-selectin may correlate with renal function. ICAM may correlate with liver function. CRP may correlate with WBC.

	psele1	icam1	tnf1	il1	crp1	troi1
got	-0.2182	0.2900*	-0.0574	0.0109	0.3111*	0.3855*
	0.0676	0.0141	0.6347	0.9284	0.0083	0.0009
	71	71	71	71	71	71
bil	-0.0657	0.1527	0.2092	0.2671*	0.3390*	0.3531*
	0.5862	0.2038	0.0799	0.0243	0.0038	0.0025
	71	71	71	71	71	71
bun	-0.1395	-0.1312	0.2594*	0.0324	0.0538	0.0454
	0.2458	0.2755	0.0289	0.7888	0.6557	0.7072
	71	71	71	71	71	71
cre	-0.2377*	-0.1777	0.4411*	0.1130	-0.0424	-0.2131
	0.0459	0.1382	0.0001	0.3483	0.7254	0.0744
	71	71	71	71	71	71
tg	-0.0635	0.0141	-0.1416	-0.0815	-0.1251	-0.0651
	0.6016	0.9078	0.2422	0.5023	0.3022	0.5921
	70	70	70	70	70	70
cho	-0.0590	-0.2166	-0.0323	-0.0220	-0.4274*	-0.3785*
	0.6275	0.0717	0.7904	0.8567	0.0002	0.0012
	70	70	70	70	70	70
wbc	0.0709	0.2065	-0.0579	0.0397	0.3458*	0.3974*
	0.5569	0.0841	0.6315	0.7423	0.0031	0.0006
	71	71	71	71	71	71

(* denotes significant Spearman correlation)

	psele2	icam2	tnf2	il2	crp2	troi2
got	0.1341	0.3223*	0.0174	-0.1230	0.3258*	0.5086*
	0.2650	0.0061	0.8856	0.3067	0.0056	0.0000
	71	71	71	71	71	71

bil	0.1093	0.4260*	0.1937	0.1096	0.2852*	0.4532*
	0.3641	0.0002	0.1056	0.3628	0.0159	0.0001
	71	71	71	71	71	71
bun	-0.2504*	0.0045	0.2984*	0.1499	0.1255	0.1923
	0.0352	0.9703	0.0115	0.2120	0.2972	0.1082
	71	71	71	71	71	71
cre	-0.2720*	0.0921	0.4835*	0.2398*	0.0271	-0.0099
	0.0218	0.4447	0.0000	0.0439	0.8225	0.9349
	71	71	71	71	71	71
tg	-0.0071	-0.2209	-0.1517	-0.2408*	-0.0687	-0.1097
	0.9533	0.0661	0.2100	0.0447	0.5721	0.3661
	70	70	70	70	70	70
cho	-0.1271	-0.2022	-0.1270	0.0442	-0.3695*	-0.2378*
	0.2945	0.0932	0.2947	0.7166	0.0016	0.0474
	70	70	70	70	70	70
wbc	0.2502*	0.2140	-0.1015	-0.1227	0.3606*	0.3565*
	0.0354	0.0732	0.3995	0.3081	0.0020	0.0023
	71	71	71	71	71	71

(* denotes significant Spearman correlation)

est to compare markers between two groups

1	Crp1	Troi1	Psele2	Icam2	Tnf2	Il2	Crp2	Troi2
	<0.0001	<0.0001	0.0201	0.0012			<0.0001	<0.0001
						0.0409		
					0.0498			
	<0.0001	0.0047	0.0492	0.0002	0.0036		<0.0001	0.0048
							0.0273	
								00177

	tg	cho	Wbc
)75		0.0034	0.0029
		0.0014	0.0120
		0.0270	

Trends:

(1) Lots of variables differed between early and late HTx groups and between infection and non-infection groups.

(2)Tnf2 (TNF-PB) differed between CAD and non-CAD groups (p=0.0498).

(3) IL2 (IL2-PB) differed between rejection and non-rejection groups (p=0.0409).

(4) Crp2 (CRP-PB) differed between long and short survivors (p=0.0273).

(5) Troi2 (Troponin-I-PB) differed between statin users and non-users (p=0.0177).

(6) CHO differed between Tapal users and non-users (p=0.0270).

四、結果成果自評

Further understanding of those markers was needed for more interpretation.

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