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Influence of Atherosclerotic Change in the Coronary Arteries of Rat Heart and Heart-lung Transplants on Plasma Lipid, Blood Glucose Levels and Platelet Aggregation

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Abstract We examined the changes in plasma lipid levels, blood glucose levels and platelet aggregation two months after transplantation in both rat heart and heart-lung transplant models. Atherosclerosis had occurred in grafted hearts in both groups by two months after the transplantation. We have previously shown that atherosclerotic changes in the grafted heart were more advanced in the heart transplantation group than the heart-lung transplantation group. Two months after the transplantation there was no significant difference in plasma lipid levels, blood glucose levels and platelet aggregation between the transplanted groups and a control group. It was concluded that plasma lipid, blood glucose levels and platelet aggregation are not related to the atherosclerotic change in the grafted heart.

Key Words : Heart transplantation, Heart-lung transplantation, Plasma lipid levels, Platelet aggregation, Chronic rejection

Introduction

Following immunosuppression, especially with regimen including cyclosporine A, long term survival of human cardiac transplant recipients has been commonly obtained. However, in the long-term, one of the major causes of mortality in heart transplant recipients is graft coronary artery atherosclerosis^(1,2). Histologically, the transplant arterial lesion is similar to nontransplant coronary atherosclerosis⁽³⁾. The etiology of this diffuse atherosclerosis appears to be immunological in nature, with both cellular and antibody participation in the intimal injury⁽⁴⁾. An especially important factor is the presence of cytotoxic B cell antibodies

which have been identified in heart transplant recipients with atherosclerosis⁽⁵⁾. Hyperlipidemia, high-density lipoprotein (HDL) decline, platelet and fibrin deposition and high glucose levels have been reported as accelerating atherosclerosis⁽⁶⁾. In patients with atherosclerosis, hyperlipidemia and hyperactive platelets are common and have been reported as important factors in progressive atherosclerosis⁽⁷⁾.

Previously we reported atherosclerotic changes of the coronary arteries in both rat heart and heart-lung transplantation models which were due to chronic rejection⁽⁸⁾. In that paper the changes in the coronary arteries in the heart transplantation group were significantly more severe than in the

heart-lung transplantation group, which may be explained by the observed difference in severity of rejection between heart and heart-lung transplants. It has been clinically reported that cyclosporine A has an platelet aggregating effect as thromboembolic complications in renal transplant recipients are increased^(9,10). However, there are few previous reports concerned with plasma lipid levels after transplantation⁽¹¹⁾. To our knowledge, there are no reports on platelet aggregation in an experimental transplantation model. Therefore in this paper we examined whether the changes in plasma lipid levels, blood glucose levels and platelet aggregation that occurred were due to the atherosclerotic change seen in the coronary arteries of rat transplantation models. Furthermore, we compared the plasma lipid levels and platelet aggregation in the heart transplantation group with those in the heart-lung transplantation group because the immunological response is weak towards heart-lung transplants⁽¹²⁾.

Materials and Methods

Experimental animals Inbred male Lewis (RT-1¹) and F344 (RT-1^{1v1})(from Seiwa Experimental Animals, Ltd., Japan) strain rats 3 to 4 months of age were used for the heart and heart-lung transplants.

Heart and Heart-lung transplantation The heterotopic heart and the heart-lung transplantations were performed to the abdominal great vessels of the recipient by a modified Ono-Lindsey's method⁽¹³⁾ and the Lee's method⁽¹⁴⁾, respectively. In all experimental models, F344 rats were used as donors and Lewis rats were used as recipients. The graft survival was monitored every day by palpating contraction of the transplanted heart through the abdominal wall. All operations were performed under light ether anesthesia. A brief discussion of each operation follows:

Heterotopic heart transplantation; The donor venae cavae and pulmonary veins were ligated and pulmonary artery and aorta were divided, then the heart was removed. The ascending aorta of the graft was anastomosed end to side to the infrarenal aorta of the recipient and the pulmonary artery was joined in a similar fashion

to the inferior vena cava.

Heterotopic heart-lung transplantation; The heart and lung were removed en block from the recipient after division of the venae cavae, pulmonary artery and aorta. The right lobes of lung were removed. The ascending aorta of the graft was anastomosed end to side to the infrarenal aorta of the recipient and a right atrial opening was anastomosed in a similar fashion to the inferior vena cava.

Immunosuppression Cyclosporine A (Sandoz Pharmaceuticals Corporation, Switzerland) was injected intramuscularly daily for twenty days after transplantation, at a dose of 10mg/kg/day. Following which no further immunosuppression therapy was given.

Plasma lipid and glucose levels Plasma lipid and glucose levels were measured two months after transplantation. Plasma lipid levels measured included triglyceride, total cholesterol and high-density lipoprotein cholesterol (HDL-cholesterol). The profiles were measured using UNIKIT Triglyceride-N, UNIKIT Cholesterol-E, UNIKIT HDL-cholesterol-N and UNIKIT Glucose-E (CHUGAI PHARMACEUTICAL CO., LTD., Jpan) after an overnight fast.

Platelet aggregation Platelet aggregation was also measured two months after transplantation. After an overnight fast, the rats were anesthetized with ether and 3.6 ml blood collected using a cardiac puncture procedure into a syringe containing 0.4 ml of 3.8% sodium citrate solution for a final volume of 4.0 ml. The solution was then centrifuged at 160×g for 10 min. The supernatant was pooled as platelet rich plasma (PRP). The remaining erythrocyte mixture was centrifuged at 1000×g for 10 min. This centrifugation produced a supernatant of platelet poor plasma (PPP) which was used to dilute the different platelet preparations of pooled PRP to about 500×10³/ml. We used the AGGREPACK-kit (Kyoto DAIICHI Kagaku Co., Ltd., Japan) for the platelet aggregation reagents and platelet aggregation was measured with AGGREGORDER II, PA-3220 (Kyoto DAIICHI Kagaku Co., Ltd., Japan). The final concentration of the adenosine diphosphate (ADP) solutions used as the platelet aggregation reagents were 1 and 3 μM. Two cuvetts containing 0.45 ml PRP solutions were incubated at 37°C for 1 min and then 0.05 ml of ADP solution was added. The reaction was observed for 5 min at 37°C. The platelet aggregation was evaluated for the maximum percentage of aggregation after addition of

1 or $3\mu\text{M}$ ADP reagent. The platelet aggregation experiment took no more than 3 hours.

Statistical analysis Data of graft survival periods are presented as the mean \pm SD. To compare differences between the heart and the heart-lung transplantation groups, the unpaired t-test was used. Results for plasma lipid levels, glucose levels and platelet aggregation are presented as the mean \pm SEM, and the Kruskal-Wallis test and the Scheffe method were used to compare the values.

Results

Graft survival Graft survival periods of both the heart transplantation group and the heart-lung transplantation group with and without immunosuppression are shown in Table 1. The mean graft survival of the heart transplantation group (n=4) was 14.3 ± 2.9 days and that of the heart-lung transplantation group (n=4) was 25.5 ± 5.4 days. Without immunosuppression the graft survival period of the heart-lung transplantation group was longer than that of the heart transplantation group, statistically ($p < 0.05$). The graft survival periods of both the heart transplantation group and the heart-lung transplantation group with immunosuppression were more than 2 months.

Plasma lipid levels The plasma lipid and blood glucose levels were measured in controls not undergoing transplantation or

receiving immunosuppression and 2 months after transplantation of both heart and heart-lung grafted rats receiving immunosuppression. The plasma levels of triglyceride, total cholesterol and HDL-cholesterol are shown in Table 2. Blood glucose levels are also shown. No significant differences was demonstrated in all measurements of plasma lipid levels and glucose level among the three groups.

Platelet aggregation Platelet aggregation was measured 2 months after the transplantation in both heart and heart-lung transplantation groups receiving immunosuppression. The platelet count in the platelet rich plasmas of all groups were uniform. Platelet aggregation was examined after the final addition of the $1\mu\text{M}$ and $3\mu\text{M}$ ADP solutions. In the control group the maximal platelet aggregation percentage was $4.9 \pm 6.3\%$ at $1\mu\text{M}$ ADP addition and $12.4 \pm 3.2\%$ at $3\mu\text{M}$ ADP addition. In the heart transplantation group, it was $7.5 \pm 3.5\%$ at $1\mu\text{M}$ ADP addition and $17.5 \pm 6.7\%$ at $3\mu\text{M}$ ADP addition. In the heart-lung transplantation group, it was $4.1 \pm 1.7\%$ at $1\mu\text{M}$ ADP addition and $12.5 \pm 3.2\%$ at $3\mu\text{M}$ ADP addition. The maximal platelet aggregation percentages of both transplantation groups were statistically close to those of the control group (Figure 1, 2).

Table 1 Graft survival periods of both heart and heart-lung transplantation groups.

Group	n	Graft survival period (days)	MST	p
<u>Without Immunosuppression</u>				
Heart Transplantation	4	10, 15, 16, 16	14.3 ± 2.9	<0.05
Heart-lung Transplantation	4	18, 25, 29, 30	25.5 ± 5.4	
<u>With Immunosuppression</u>				
Heart Transplantation	6	all >60		
Heart-lung Transplantation	6	all >60		

n=number of animals. MST=Mean survival time in days (Mean \pm S.D.).

Table 2 Plasma lipid levels and glucose levels

Group	n	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	HDL-Cholesterol (mg/dl)	Glucose (mg/dl)
Control	7	49.9±5.2	46.6±2.3	37.7±5.3	94.6±3.2
Heart Transplantation	6	65.0±6.7	45.3±7.0	34.8±5.3	95.5±6.9
Heart-Lung Transplantation	7	67.1±7.1	47.4±5.1	34.7±5.4	78.3±5.7

n; Animal number, HDL; high density lipoprotein, Mean±SEM.

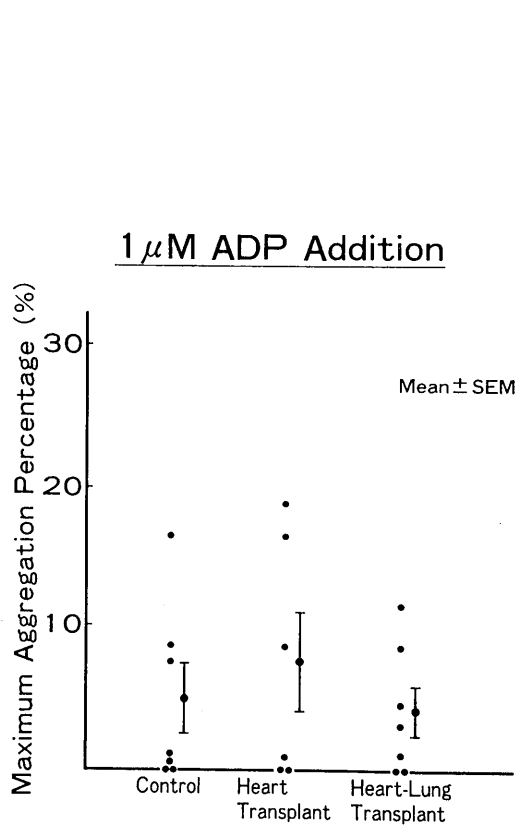


Figure 1; Platelet maximum aggregation percentage with 1 μ M ADP in the control group (n=7), the heart transplantation group (n=6) and the heart-lung transplantation group (n=7). No significant difference is seen among the three groups.

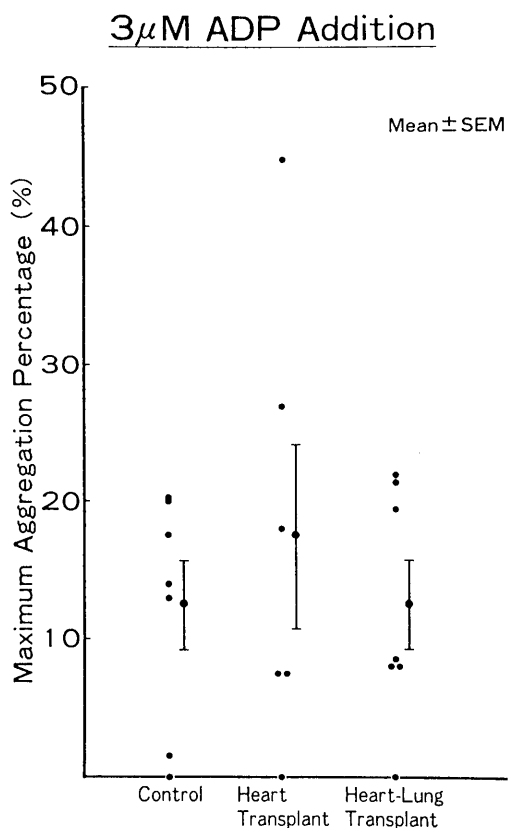


Figure 2; Platelet maximum aggregation percentage with 3 μ M ADP in the control group (n=7), the heart transplantation group (n=6) and the heart-lung transplantation group (n=7). No significant difference is seen among the three groups.

Discussion

The most serious long-term problem in human cardiac transplantation is atherosclerosis in the coronary arteries of the grafted heart^(1,2). In some cases the atherosclerosis rapidly accelerates and is fatal. An additional danger is that recipients have no chest pain at the time of serious coronary artery atherosclerosis because the grafted heart is denerved. Therefore the optimal time to perform a coronary angiography examination is difficult to determine. In the clinic, many risk factors such as hyperlipidemia, hyperglycemia and hypertension have been shown to accelerate the development of atherosclerosis^(6,7). In rabbit experimental model hyperlipidemia accelerated atherosclerosis in the graft⁽¹¹⁾. Several papers reported that cyclosporine A caused high platelet aggregation^(9,10). However, the main cause of atherosclerosis of the grafted heart is the immune response^(4,7).

We previously showed that atherosclerotic changes occur in the coronary arteries of grafted hearts using same model⁽⁸⁾. Those changes were similar to those of nontransplanted atherosclerosis⁽³⁾. The grade of atherosclerotic change of the grafted heart was previously been shown to be more advanced in the heart transplantation group than the heart-lung transplantation group⁽⁸⁾. The lung grafts may be more antigenically active than heart allografts because of the presence of lymphoid aggregates, bronchus-associated lymphoid tissue (BALT)⁽¹⁵⁾. However, plasma lipid levels, blood glucose levels and platelet aggregation of both the transplantation groups were similar to the control group. Therefore it is concluded that the atherosclerotic change in grafted heart is not related to changes in plasma lipid or blood glucose levels, platelet aggregation.

Our experiment dose not address the effect of long-term immunosuppression on plasma lipid levels, blood glucose levels and platelet aggregation. These effect are not currently understood. Atherosclerosis had developed by one month after the transplantation in both groups in our study⁽⁸⁾. In similar transplantation study using the ACI to LBN rat

combination atherosclerosis was detectable as early as twenty days after the transplantation⁽¹⁶⁾.

It has been suggested that anti-platelet agents have an effect against the development of atherosclerosis^(16,17). Our results showed no increase in the tendency of circulating platelets to aggregate in recipients of heart or heart-lung transplants receiving cyclosporine A. However, the inner surfaces of the coronary arteries of the grafted hearts were irregular and platelet might more easily adhere and lead to further fibrin deposition. Anti-platelet drugs may therefore have no effect on the development of atherosclerosis but might reduce its severity and the risk of thrombosis once it is established.

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