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Effect of Immunomodulatory Artificial Blood Exchange (IABX) on Guinea Pig-to-Rat Heart Xenografts

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ABSTRACT

The aim of this study was to assess the effectiveness of pregraft immunomodulatory artificial blood exchange (IABX) in a guinea pig-to-rat xeno discordant heart transplantation, using an artificial blood (FC43 emulsion: The Green Cross, Japan) in exchange for a large volume of whole blood to remove humoral immune factors en bloc from the recipient rat. In the rats treated with IABX, rhythmic beating of the grafted heart was maintained for 2 hr, whereas the untreated heart beat lasted for only 15.2 ± 5.2 min (n=6). In the graft hearts treated with IABX, no pathologic changes such as multiple coronary thromboses due to hyperacute rejection (HAR) were observed. Humoral immune factors (natural-IgM titer, ACH50 and CH50 complement activities, platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen serum concentration), which are thought to contribute to HAR, decreased significantly following the IABX treatment.

We conclude that IABX is an efficient method for prolonging the survival time of guinea pig heart xenografts by inhibiting thrombus formation in the xeno-graft heart. It was confirmed that IABX could remove recipient humoral immune factors en bloc.

Key words: *Xeno discordant transplantation, Hyperacute rejection, Artificial blood, Blood exchange*

The existence of natural antibodies, serum complement activities, coagulation factors and other humoral immune factors are part of a well-established phenomenon in discordant xenograft transplantations, and commonly lead to hyperacute rejection (HAR)[1,21,23,24)]. The aim of this study was to assess the effectiveness of a pregraft immunomodulatory artificial blood exchange (IABX) on a guinea pig-to-rat cardiac transplantation. This was accomplished by using a perfluorochemical-based red blood cell substitute (FC43 emulsion: The Green Cross, Japan) in exchange for a large volume of whole blood to remove humoral immune factors en bloc from the rat.

MATERIALS AND METHODS

Animals

Mature male guinea pigs (mean ± S.D. of mean body weight: 312 ± 21.5g) of 8–10 weeks of age and mature male Wistar rats (mean ± S.D. of mean body weight: 356 ± 28.4g) of 9–12 weeks of age (Hiroshima Experiment, Japan) served as cardiac xenograft donors and recipients, respectively. The animals were kept in cages in a laboratory animal house. The guinea pigs were maintained on a diet of RC4 pellets (Orientaly East, Japan) and the Wistar rats were maintained on MF pellets (Orientaly East, Japan). Free access to water and food were allowed at all times. The temperature and lighting were regulated to maintain a temperature of 20–25 °C and a 12–14hr light cycle / day (lights on 07.00hr-). All the animals received humane care in compliance with the "Principles of Laboratory Animals Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication no. 8623, revised 1985).

Experimental procedures

Donor animals.

The guinea pigs (GP) were initially anesthetized with ether, weighed and then given a single 1.0ml/kg intraperitoneal injection of sodium pentobalbital (Nembutal: Abbott Lab., U.S.A.). The GPs were placed in a supine position, and a median sternotomy was performed. The right and left superior vena cava were then ligated with

4–0 silk. Next, cold (4°C) crystalloid potassium cardioplegia (GIK solution: K^+: 25mEq/L; HCO_3: 4.4mEq/L; pH 7.78; 350 mOsm) was perfused retrogradely via a catheter inserted through the root of the inferior vena cava at a dose of 0.1 ml/kg. The left atrium was ligated with 4–0 silk, and the pulmonary artery and aorta were transected 3–4 mm above their origins in the heart. After perfusion of the ventricles and atria with a saline solution containing 200 U/ml of heparin, the donor heart was placed in a saline bath at 4°C for about 30–45 min.

Recipient animals.

The Wistar rats (WR) were anesthetized and placed in a supine position. A midline neck incision was made, and the trachea was exposed. Then a 14 gauge catheter (internal diam. 1.75mm; outer diam. 2.1mm; Terumo, Japan) was inserted into the trachea. After this procedure, the respiration of the rat was controlled by a ventilator (EVM-50A: AIKA, Japan) at a tidal volume of 2.5 ml and a rate of 80/min with 100% O_2. The right femoral artery and vein were then exposed in the right inguinal region. Two 24 gauge polypropylene catheters (int. diam. 0.47mm; outer diam. 0.67mm; Terumo, Japan) were inserted into the femoral artery and vein.

Immunomodulatory Artificial Blood Exchange (IABX).

Isovolumetric blood exchange was performed with a novel, specially constructed apparatus[6]. A diagram of this circuit is shown in Fig. 1. The blood substitute (FC43 emulsion; Table 1) and saline were warmed to 38°C and oxygenated with 5% CO_2-95% O_2 mixture gas in a bubble oxygenator. Then the FC43 emulsion was infused via the venous catheter at a rate of 1 ml/min continuously by a JMS infuser M-04 pump (JMS, Japan), whilst the blood was simultaneously removed at the same rate from the arterial catheter by the same pump. The arterial effluent was collected serially into plastic tubes kept in a cooler box at 0°C, and the hematocrit was measured every 5 min in these serial samples. When the hematocrit fell below 20% in experimental group III and 10% in experimental group IV, the exchange procedure was discontinued. The final hematocrit was confirmed by the last blood sample. The blood pressure and heart rate were also monitored at 5 min intervals with a type 45363 pressure transducer connected to the arterial catheter, and the readings were displayed on a Multisuper type 2F21 recorder (SAN-EI Instrument, Japan). If the blood pressure fell below 70mmHg, the exchange procedure was also discontinued. The circuit was composed of polyethylene tubes (int. diam. 1.75mm; outer diam. 2.1mm; Terumo, Japan). An air filter (Pole filter: max. pore size 0.2μm; Mera, Japan) was placed in the affluent line to the oxygenator to prevent bacterial infection. The total volume of the circuit was about 2 ml. The blood exchange continued until the target hematocrit (%) was reached. At the end of the exchange, the WR were given a supplementary infusion of 1.5 ml FC43 emulsion 100g^{-1} body weight to aid survival[31]. The overall exchange usually lasted 20–30 min, depending on the final target hematocrit.

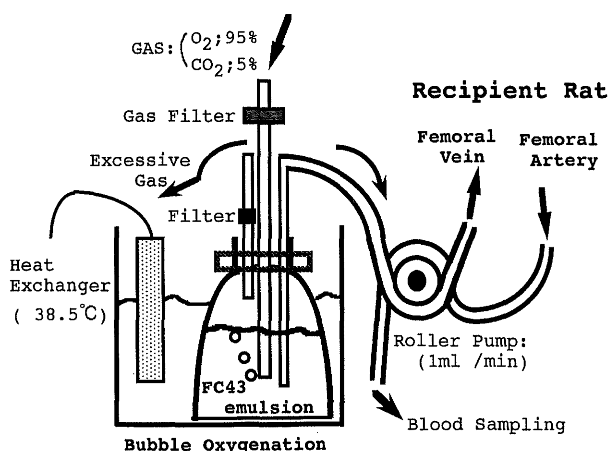


Fig. 1. Diagram of the blood exchange apparatus

Heart transplantation.

Heterotopic heart transplantation was performed using a modified Cuff technique, according to the method of Heron[7]. After isovolumetric blood exchange, the recipient WR was maintained under anesthesia and placed in a supine position. The right jugular vein and common carotid artery were then exposed and dissected free. The pulmonary artery of the graft heart was connected to the right jugular vein of the recipient WR with a 12 gauge catheter (2mm long; outer diam. 1.65mm; Portex, U.K.). The innominate artery of the graft heart was connected to the right common carotid artery of the recipient WR with a 16 gauge catheter (2mm long; outer diam. 1.02mm; Portex, U.K.). Within a few minutes following reperfusion, the xeno-grafted heart resumed its sinus rhythm. The operative duration ranged from 20 to 30 min, with a success rate of approximately 90%. The total ischemic time varied between 45 to 60 min.

Analytical procedures

Graft heart beating time.

The graft heart beating time (GH-BT) was measured by direct observation when the neck surgical fields were opened.

Blood samples.

Arterial effluent samples were collected into

Table 1. Composition of FC43 emulsion, structural formulae of FC43 (perfluorotributylamine) and Pluronic F-68 (polyxypropylene-polyxyethylene-copolymer)

Composition of FC43 emulsion	
FC43 (perfluorotributylamine)	20 w/v%
Pluronic F-68 (polyxypropylene-polyxyethylene-copolymer)	2.56w/v%
NaCl	0.60 w/v%
KCl	0.034w/v%
$MgCl_26H_2O$	0.043w/v%
$CaCl_22H_2O$	0.036w/v%
$NaHCO_3$	0.210w/v%
Glucose	0.180w/v%
Hydroxyethyl starch	3.0 w/v%

in pyrogen-free distilled water, autoclave-sterilized.

Structural formula of FC43(perfluorotributylamine)

CF_3-CF_2-CF_2-CF_2-N-CF_2-CF_2-CF_2-CF_3
CF_3-CF_2-CF_2-CF_2/

Structural formula of Pluronic F-68 (polyxypropylene-polyxyethylene-copolymer)

$HO(CH_2CH_2O)_a(CHCH_2O)_b(CH_2CH_2O)_cH$
CH_3

Physiolochemical properties of FC43 emulsion: Osmotic pressure, 309 mOsm; Oncotic pressure, 395 mmH_2O; Weight-average particle size, ca.0.1μm, freed from 0.6μm or greater particles. Pluronic F-68 is a non-ionic surfactant, added to emulsify FC43.

plastic tubes before and after the blood exchange procedure, but prior to heart transplantation. For immunologic analysis, the samples were centrifuged at 2500 r.p.m. for 10 min at –4°C to obtain the serum. The serum was then stored at –70°C and, in principle, the frozen serum was defrosted only once prior to the assay. For coagulation analysis, the samples were collected into plastic tubes containing sodium citrate, and the serum was obtained by the same procedure described above.

Hematocrit & platelet counts.

The hematocrit was measured automatically by a HITACHI MC-200 microhematocrit centrifuge (Hitachi, Japan). The platelet count and the measurement of other blood cells were performed automatically by a Celltac α Counter (Nihon Kohden, Japan). The samples were diluted accordingly using Isotonic III solution.

Complement hemolysis assay (CH50, ACH50).

The CH50 (classical pathway assay) was measured by the microtiter method according to Mayer[18]. ACH50 (alternative pathway assay) was measured according to the method of Platts-Milles and Ishizuka[25] with a slight modification in that sheep erythrocytes were substituted as target cells.

IgM titer.

The IgM titer was measured by the general radial immunodiffusion (RID) method[17] using a Binding Site RID kit (The Binding Site Limited, England).

Examination of blood coagulation factors.

The prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen serum concentration (FBG) were measured automatically by a CA-5000 automated blood coagulation analyzer (Toa Medical Electronics, Japan) as indices for coagulation activity. This method quantifies clots by detecting scattered light intensity (percentage test end-point detection method)[22].

Histological examination.

The xeno-grafted hearts were removed shortly before they stopped. For electron microscopic examination, small pieces of the left ventricular wall were excised from grafted hearts treated with or without IABX. These tissues were fixed with 3% phosphate-buffered glutaraldehyde for 2hr and post-fixed with 1% phosphate-buffered OsO_4 for 1hr. Then they were dehydrated through a graded alcohol series, embedded in epoxy resin, and thin sections cut with a Porter-Blum ultramicrotome equipped with glass knives. The sections were stained with uranyl acetate and lead citrate, and finally observed with a Hitachi H-7000 electron microscope (Hitachi, Ja-

pan). Additional tissues were fixed with 10% formalin, embedded in paraffin, and stained with hematoxylin / eosin for light microscopy.

Experimental design.

The animals were divided into four groups. Group I (control) consisted of 6 guinea pig-to-rat hearts transplanted in untreated recipients. Group II (saline exchange control) consisted of 6 animals in which the effect of blood exchange with saline was studied to the limit of hemodynamic parameters. Group III (partial exchange with FC43 emulsion; Ht=11–20%) and group IV (total exchange with FC43 emulsion; Ht≦10%) examined the effects of IABX on guinea pig-to-rat heart xenograft beating time in 9 and 10 animals, respectively.

Statistical Analysis.

Differences in the graft heart beating times were compared using the Wilcoxon rank-sum test, and were considered to be statistically significant if p values were less than 0.01. For the blood examinations, comparisons were made between values before and after the blood exchange with paired Student's t test. Differences were considered to be statistically significant if p values were less than 0.01. Comparisons were also made between the saline exchange group and IABX groups in every reduction ratio with unpaired Student's t test. Differences were considered to be statistically significant if p values were less than 0.01.

RESULTS

Graft heart beating time.

Nineteen rats were eligible for the IABX study. The graft heart beating times (GH-BT) in the 4 different experimental groups are shown in Table 2. The untreated rats (group I; Ht=45.5±5.8%) showed a hyperacute rejection of the xenograft within 15.2±5.8 min. Rats which had their whole blood exchanged to the limit of hemodynamics with saline (group II; Ht=29.8±3.2%) did not have a significantly different graft heart beating time (GH-BT=13.7±3.8 min, NS vs group I). In group III, which was treated by a partial exchange with FC43 emulsion (Ht=15.0±3.3%), the graft heart beating time was prolonged to 166.9±27.2 min (p=0.0015 vs group I, p=0.0027 vs group II). In group IV, which was treated by a total exchange with FC43 emulsion (Ht=6.2±2.4%), the graft heart beating time was prolonged to 151.6±25.1 min (p=0.0011 vs group I, p=0.0011 vs group II). There were no significant differences in the graft heart beating time between groups III and IV.

Blood examinations.

In the saline exchange group and the two IABX groups, the values of the humoral immune factors (platelet count, CH50 , ACH50, IgM titer, PT, APTT and FBG; expressed as a mean±SD) after the blood exchange but before the transplant were compared to the values before the blood exchange (Table 3.). Furthermore, the reduction ratio after the blood exchange procedure was calculated by the following equation, and expressed as a percentage (mean±SD): reduction ratio after blood exchange = (value after blood exchange / value before blood exchange) ×100%.

In group II (saline exchange to the limit of hemodynamics), 5 rats were examined. The values of the platelet count, CH50, ACH50, IgM titer and FBG after the blood exchange decreased significantly (p<0.01) as compared with the values before the blood exchange. In contrast, there were no significant differences (p>0.05) in the values for the PT and APTT before and after the blood exchange. The ratio after the blood exchange for the hematocrit decreased from 100% to 64.1±6.3%. The reduction ratio after the blood exchange was calculated in the platelet count (79.0±4.7%), CH50 (64.6±9.1%), ACH50

Table 2. Graft heart beating time (GH-BT) in each group

Groups	No. parts	Blood exchange procedure	Ht (%) Mean±SD	Graft heart beating time: Individual results (min)	Graft heart beating time: Mean±SD	P value*
I	6	—	45.5±5.8	9, 11, 12, 18, 18, 22	15.2± 5.2	
II	6	Saline exchange to the limit of hemodynamics	29.8±3.2	9, 9, 14, 16, 17, 17	13.7± 3.8	NS vs. I
III	9	Partial exchange with FC43 emulsion (Ht=11–20%)	15.0±3.3	125, 133, 152, 155, 167, 181, 189, 194, 203	166.9±27.2	<0.01 vs. I <0.01 vs. II
IV	10	Total exchange with FC43 emulsion (Ht≦10%)	6.2±2.4	102, 132, 135, 144, 150, 156, 157, 178, 180, 182	151.6±25.1	<0.01 vs. I <0.01 vs. II NS vs. III

* Wilcoxon rank-sum test

Table 3. Changes in humoral immune factors by blood exchange in group II, III and IV

Group	Procedure	HT (%)	PLT ($\times 10^4$/mm^3)	CH50 (U/ml)	ACH50 (U/ml)	IgM (mg/liter)	FBG (mg/dl)	PT (sec)	APTT (sec)
II	before blood exchange	46.6 ±1.9	98.0 ±5.6	52.1 ±3.1	292.2 ±29.4	1108.7 ±81.0	191.6 ±15.5	14.0 ±0.1	52.4 ±3.7
								NS	NS
	after blood exchange	28.9 ±2.6	77.5 ±7.9	33.5 ±3.5	174.8 ±20.6	901.7 ±87.3	166.2 ±18.9	13.4 ±0.6	48.8 ±3.8
III	before blood exchange	48.2 ±3.6	109.7 ±16.3	55.9 ±5.3	287.7 ±28.4	1117.7 ±78.7	209.4 ±38.7	14.1 ±0.2	48.6 ±5.1
	after blood exchange (IABX)	15.0 ±2.7	37.2 ±6.4	<7.7 ±3.2	50.6 ±17.3	603.3 ±173.2	12.8 ±6.3	25.4 ±5.1	86.0 ±14.4
IV	before blood exchange	46.0 ±3.2	103.2 ±7.3	53.9 ±5.8	209.8 ±59.4	1098.3 ±80.2	205.0 ±44.9	14.0 ±0.6	48.4 ±6.2
	after blood exchange (IABX)	7.6 ±1.5	17.8 ±5.5	<6.2	18.0 ±6.8	321.2 ±70.2	<10	44.6 ±16.8	149.0 ±28.5

Ht, hematocrit; PLT, platelet count; IgM, Immunoglobulin M titer; FBG, fibrinogen serum concentration; PT, prothrombin time; APTT, activated partial thromboplastin time. Every value expressed as a mean ± SD. All of the values after the blood exchange decreased or increased significantly (p<0.01) as compared with values before the blood exchange, except the values for PT and APTT in group II.

(60.1 ± 7.5%), IgM titer (81.5 ± 4.5%) and FBG (86.6 ± 3.2%). Each of these values decreased significantly (p<0.01) after the blood exchange as compared with values before the blood exchange. On the other hand, there were no significant increases in the ratio for the PT (95.4 ± 4.3%) and APTT (93.9 ± 6.7%) after the blood exchange.

In group III (partial exchange with FC43 emulsion; Ht=11–20%), 5 rats were examined. All values of the humoral immune factors (platelet count, CH50, ACH50, IgM titer, FBG, PT and APTT) decreased or increased significantly (p<0.01) after the blood exchange as compared with the corresponding values before the blood exchange. Moreover, the reduction ratio after the blood exchange for the hematocrit decreased from 100% to 31.1 ± 5.3%. There were marked decreases in the reduction ratio after the blood exchange for the platelet count (34.3 ± 5.9%), CH50 (<14.0 ± 6.2%), ACH50 (18.2 ± 8.3%), IgM titer (53.3 ± 12.3%) and FBG (<6.1 ± 1.5%). There were also marked increases in the reduction ratio after the blood exchange for the PT (179.1 ± 33.9%) and APTT (178.7 ± 35.0%). All of the ratios after the blood exchange showed significant differences (p<0.01) as compared with ratios before the blood exchange (= 100%).

In group IV (total exchange with FC43 emulsion; Ht ≦ 10%), 5 rats were examined. All values of the humoral immune factors (platelet count, CH50, ACH50, IgM titer, FBG, PT and APTT) decreased or increased significantly (p<0.01) after the blood exchange as compared with corresponding values before the blood exchange. Furthermore, the reduction ratio for the hematocrit after the blood exchange decreased from 100% to 17.4 ± 3.6%. There were marked decreases in the reduction ratio after the blood exchange for the platelet count (17.0 ± 4.6%), CH50 (<11.6 ± 1.2%), ACH50 (6.5 ± 3.0%), IgM titer (29.2 ± 5.6%) and FBG (<5.1 ± 1.0%). There were also marked increases after the the blood exchange in the reduction ratios for the PT (320.1 ± 121.1%) and APTT (317.1 ± 90.8%). All of the ratios after the blood exchange showed significant differences (p<0.01) from those before the blood exchange.

Comparisons between the saline exchange group and the IABX groups about the reduction ratio in all humoral immune factors.

In every reduction ratio between the IABX groups and the saline exchange group there was a significant difference (p<0.0005, except in IgM between group II and III; p<0.001) (Fig. 2).

Histological examinations.

Light microscopic examination.

Light microscopic examination of the xenografted hearts in groups I (Fig. 3a) and II shortly before the heart stopped beating showed that the coronary vessels were markedly dilated and filled with erythrocytes. Erythrocytic and thrombocytic thrombi were frequently observed in the coronary vessels, and the myocardial cells in the left ven-

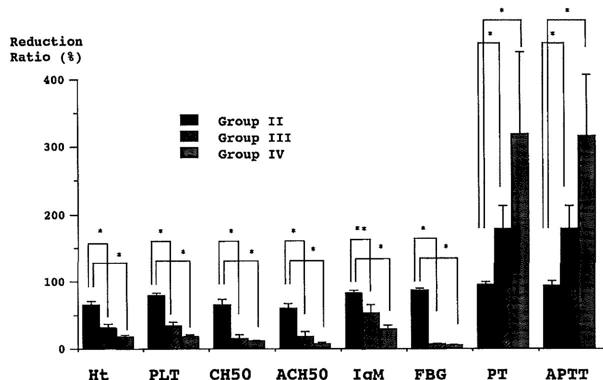
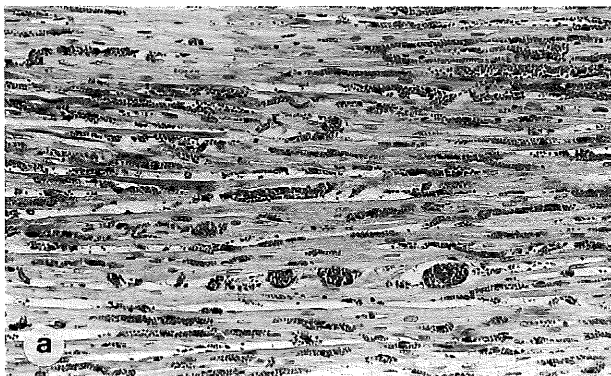


Fig. 2. Reduction ratio after blood exchange in the saline exchange group and IABX groups. Every reduction ratio in the IABX groups showed a significant difference compared with the ratio in the saline exchange group (*p<0.0005; **p<0.001). For abbreviations and explanations, see Table 3.



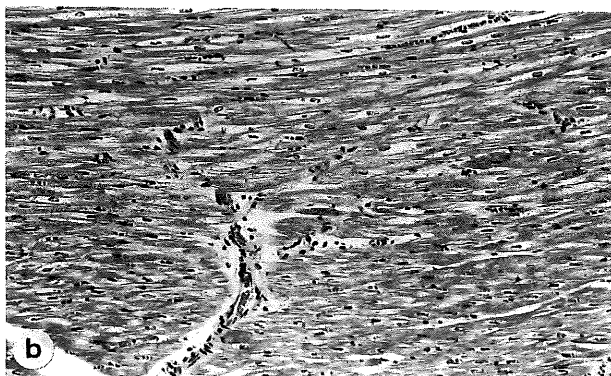


Fig. 3. Light microscopic examination of grafted hearts in group I (a; ×270) revealed that erythrocytic and thrombocytic thrombi were recognized frequently in coronary arteries and veins shortly before the heart stopped. Degenerative and necrotic myocardial cells containing pyknotic nuclei and eosinophilic cytoplasm were scattered in the left ventricular wall. On the other hand, these changes were not observed in animals of group IV treated with IABX (b; ×270) shortly before the grafted heart stopped.

tricular wall were markedly stretched and edematous. Degenerating, necrotic myocardial cells containing pyknotic nuclei and eosinophilic cytoplasm were also scattered throughout the left ventricular wall. The interstitium was markedly edematous, with diffuse extravasation. In contrast, in the IABX treated groups, vascular dilatation and thrombus formation were not observed in the grafted heart (Fig. 3b).

Electron microscopic examination.

Electron microscopy of the grafted heart in groups I and II revealed that many thrombocytes had adhered to the endothelial cell surfaces by 10 minutes after the transplantation (Fig. 4a). At this stage, the capillary endothelial cells appeared to be stretched. In addition to the adhering thrombocytes, vacuolar changes were frequently recognized in these endothelial cells. By the time of rejection, most endothelial cells had degenerated markedly and contained pyknotic nuclei (Fig. 4b). The cytoplasm of these degenerated cells became discontinuous and many thrombocytes were in direct contact with the basement membrane of these capillary vessels. Extravasations were also frequently observed around these damaged capillaries. Endothelial cells in the coronary arteries and veins showed severe degenerative changes with large hydropic vacuoles, swollen cytoplasmic projections and bleb formation. The arterial and venous luminae were frequently filled with aggregated thromobocytic thrombi (Figs. 4c and 4d).

In the IABX treated groups, thrombocyte adhesion and erythrocytic or thrombocytic thrombi were not found, and the endothelial cells remained well-preserved until 2 hours after transplantation (Figs. 5a and 5b).

DISCUSSION

Treatment by human organ allotransplantation is severely restricted due to the lack of donor organs. Thus, experimental xenotransplantation and the feasibility of using substitute donor organs from nonhuman sources have been studied[1,4]. However, no effective immunosuppressive methods have been developed for xenotransplantation. In xenotransplantation, the recipient humoral immunity constitutes the initial phase of rejection; this immunity is not inhibited by cellular immunosuppressive agents used in clinical allotransplantation. Humoral immunity is activated even in unsensititized xenotransplantation subjects[23,24]. Species combinations in which rejection occurs most severely and within only a few hours are considered to be xeno-discordant combinations[3]. This type of rejection is termed HAR, and the graft is rejected immediately because of multiple thrombi in the vessels. Although the humoral immunosuppressive methods presently available have

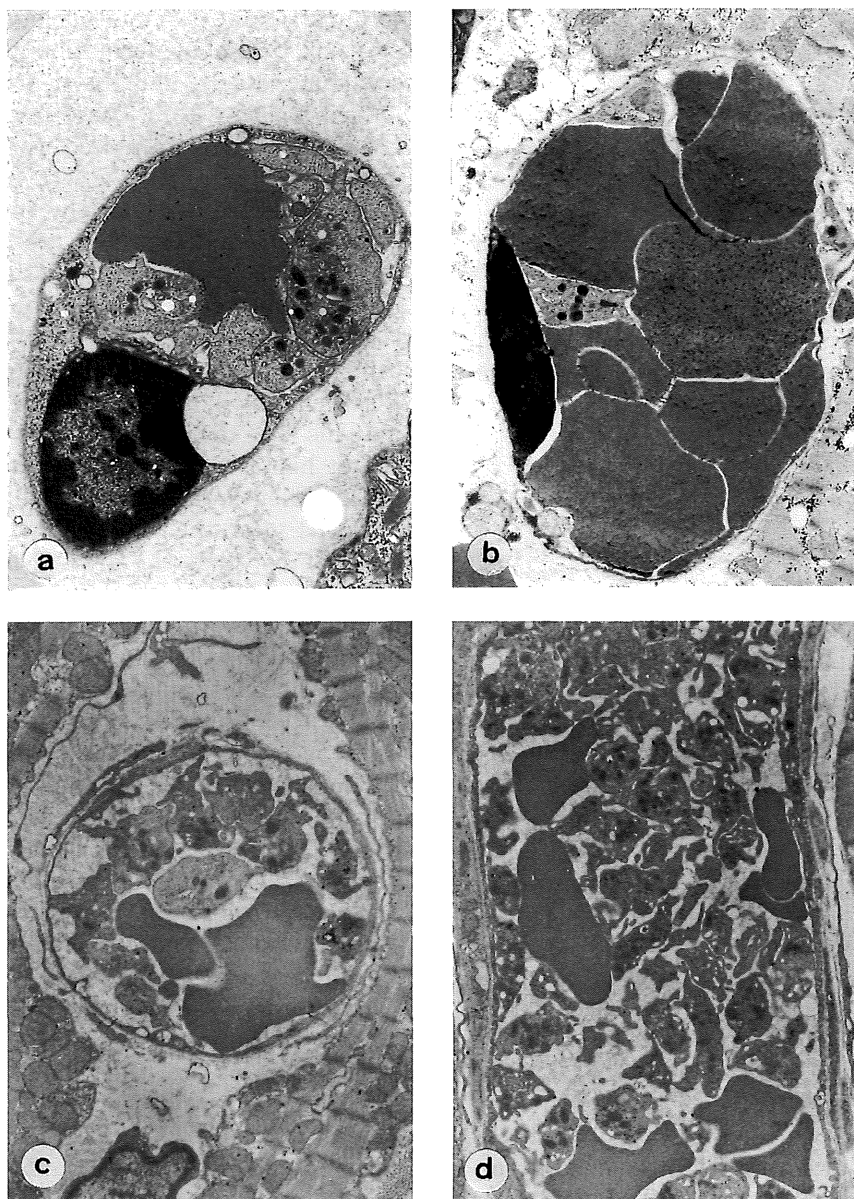


Fig. 4. Electron microscopic findings of the grafted heart in group I showed that numerous thrombocytes adhered to the endothelial cell surface at 10 min after transplantation (a; ×5900). At the time of rejection, capillaries were filled with erythrocytes and thrombocytes, and endothelial cells were degenerative and necrotic (b; ×5900). In the arteriole (c; ×4400) and venule (d; ×4200) numerous thrombocytes aggregated and formed thrombocytic thrombus.

some effect on graft survival, there are no adequate methods to counteract the HAR[12,14,21,30]. However, if grafts from discordant species could be used, then the utility of treatment by transplantation would be extended.

IABX is a new immunosuppressive technique which removes a large volume of humoral immune factors en bloc by exchanging whole blood for artificial blood. In this study we used FC43 emulsion as a red cell substitute (RCS). FC43 emulsion was developed as a pefluorochemical (PFC)-based RCS and has been studied since the 1970s. FC43 emulsion is one of the most biochemically stable PFC-based RCS *in vivo*[5,28,32], and its pH, osmotic pressure, and electrolyte composition are ideally suited for administration *in vivo*[19].

In this study, we used a xeno-discordant heart transplantation model to induce HAR. In this model, the graft heart beating stopped almost immediately because of multiple thrombi in the coronary arteries and veins. Therefore, we tested the effectiveness of IABX for extending the GH-BT in this model. As shown in Table 2, the xenografted hearts ceased beating by about 15 min after reperfusion in the untreated and saline exchange groups, whereas the heart beat was maintained in the IABX groups for 150–160 min after reper-

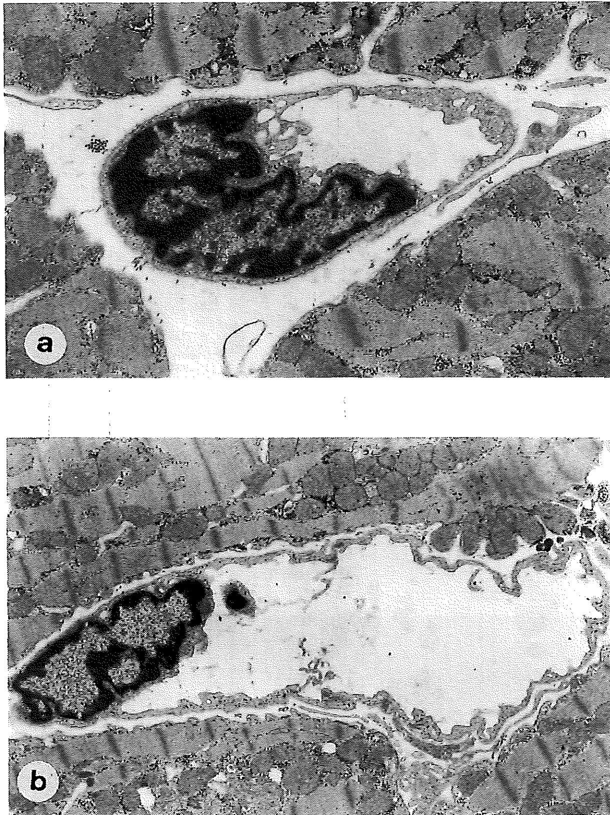


Fig. 5. Electron microscopic findings of the grafted heart treated with IABX (group IV) showed that capillary endothelial cells of arterial side (a; ×5800) and venous side (b; ×4400) were well preserved and thrombus formation could not be found at 2 hr after transplantation.

fusion. These results indicate that IABX was effective as a pregraft immunomodulatory procedure in prolonging the GH-BT in this xenotransplantation model, and that the use of artificial blood substitutes may be effective for removing humoral immune factors in cases of large blood volume exchange. It was impossible to decrease the hematocrit below about 30% in the saline exchange group because of hemodynamic limitations, and humoral immune factors were not reduced sufficiently.

Although the mechanisms underlying the HAR are not completely understood, humoral immune factors have been thought to contribute substantially to the HAR[1,21,23,24]. Our data confirm these immune factors can be significantly attenuated by IABX, and thus would lessen the extent of HAR.

Morphologically, endothelial cell damage and numerous thrombi were found in the coronary vessels of grafted hearts in groups I and II. Therefore, these myocardial cells were probably injured by the severe acute ischemia caused by the stoppage of the coronary circulation[10,15]. However, in grafted hearts treated with IABX (groups III and IV), neither endothelial cell damage nor thrombus formation were found in the coronary vessels, and the heart beating was prolonged for over 2 hr. These findings indicate that IABX treatment inhibited thrombus formation, and had a protective effect on the endothelial cells of the grafted heart by removing the humoral immune factors which are activated in HAR.

Whilst under general anesthesia the recipient rats did not receive any additional infusions after the blood exchange procedure for about 2 hr, except for the supplementary infusion of 1.5 ml FC43 emulsion $100g^{-1}$ body weight to aid survival. This was done so as not to change the initial target hematocrit. The arterial blood pressure of the recipient rats fell gradually from about 2 hr onwards after the blood exchange. Thus there is a possibility that the grafted hearts stopped under the influence of low perfusion pressure in the recipient rats. In addition, it has been reported by some investigators that FC43 emulsion has some immunosuppressive effects[2,13,20]. Both of these factors must be considered in assessing the influence of blood substitutes on HAR.

IABX has an advantage over conventional plasmapheresis for immune-absorption[9,11,26,30] because IABX can remove lymphocytes, platelets[16,27,29] and other unknown immunoreactive mediators to HAR en bloc. This procedure is simpler and easier to perform than plasmapheresis. Moreover, there is no risk of infection from using human origin blood substitutes.

As new immunosuppressive agents are developed in the future, the administration of an immunosuppressant combined with IABX may prove to be more effective because this procedure can remove humoral immune reactive factors[8] and administer the agent in large quantities (in the case of a rescue therapy) at the same time.

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