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INTRAVASCULAR COAGULATION IN HYPERACUTELY REJECTED LIVER XENOGRAFTS

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In order to investigate whether the cessation of blood flow in a rabbit-to-dog liver graft is due to enzymatically induced intravascular coagulation in the transplant, 2 experiments were carried out. The gradient of coagulation factors across the graft was measured during the rejection. No consumption of factors V, VII, and X was found. There was retention of 11% of inflowing fibrinogen, and prolongation of calcium clotting time by 30 sec, and thrombin time by 60 sec. Nonetheless, blood flow stopped in the organ within 5—7 minutes.

Vascularized organs transplanted between genetically divergent species undergo hyperacute rejection. The time of survival of the graft depends on the degree of genetic similarity or dissimilarity between the donor and the recipient. In most cases rejection takes place within minutes after revascularization of the graft. The hemodynamic effect of rejection consists in rapidly increasing vascular resistance and decreasing blood flow through the transplanted organ. The immunological mechanism of hyperacute rejection, as well as blood flow cessation, remain obscure. The generally accepted opinion is that an instantaneous antigen-antibody reaction between the preformed circulating antibodies of the recipient and erythrocyte, leukocyte and/or endothelial cell surface antigens of the donor precipitate a coagulopathy. Natural hemagglutinins and lymphoagglutinins of the recipient species directed against antigens on the cellular membrane of erythrocytes, lymphocytes, and endothelial cells initiate the intravascular immunological reaction. Antigen-antibody complexes attract polymorphonuclear cells and thrombocytes via C'activation. Aggregated platelets as well as leukocytes capable of inducing clotting appear. Platelet aggregates may also plug the microvasculature, decreasing blood flow. Vasoactive substances released from disintegrated platelets and leukocytes damge the capillary wall, increasing its permeability, thus creating additional reasons for increased vascular resistance. It remains then to be clarified whether the cessation of blood flow in a xenograft is

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primarily due to: a) immunological injury to the endothelial cells with subsequent disintegration of cells, increased capillary permeability, edema of tissue, and compression of the microvasculature followed by intravascular coagulopathy, or, b) simply to immunologically mediated coagulopathy that may lead to the devascularization and destruction of a transplanted organ. If this second contention were true, antithrombotic therapy should prove to be of prime importance for prevention of hyperacute rejection.

The purpose of the present study was to investigate whether the cessation of blood flow in a rabbit-to-dog liver graft is due to the enzymatically induced intravascular coagulation in the transplant.

MATERIAL AND METHODS

Twenty-one experiments were carried out in 3 groups. In group 1 of 15 dogs and rabbits, rabbit liver was transplanted to the dog neck vessels (Olszewski et al., 1973). In group 2 of 6 rabbits, 3 liver allografts were performed with anastomosis to the recipients absominal aorta and the IVC. In group 3, of 6 dogs a liver allograft was made with anastomosis to the recipient neck vessels.

Blood samples were taken for coagulation and fibrinolysis studies: a) from the artery before the revascularization of the graft, b) from the affluent and effluent blood at 5 min after reestablishment of perfusion. Blood flow through the graft was estimated in the 1st and 5th minute of perfusion.

The following coagulation tests were performed:
1. Plasma calcium clotting time.
2. Prothrombin time (Quick, 1951).
3. Factor VII and X (Koller et al., 1951).
4. Factor V (Wolf, 1953).
5. Thrombin time with 0·2 ml of plasma and 0·1 ml of thrombin.
6. Fibrinogen level.
7. Euglobulin lysis time.
8. Soluble fibrin monomer complexes (Lipinski et al., 1968).
9. Platelet counts.

RESULTS

Group 1. The data are summarized in Table 1. The rejection time as measured by blood flow decrease below 0·05 ml/g/min ranged between 5 and 7 minutes. At 5 minutes after revasculatization of the graft 70%

Table 1. Coagulation and fibrinolysis in affluent and effluent blood in a rabbit-to-dog liver xenograft (a mean of 15 experiments)

	Affluent blood	Effluent blood
Platelets in cu mm	$293 \cdot 000 \pm 84 \cdot 900$	$65 \cdot 000 \pm 16 \cdot 400$
Calcium clotting time (sec)	$47 \pm 7 \cdot 5$	$61 \pm 5 \cdot 0$
Prothrombin time (%)	$100 \pm 1 \cdot 5$	$88 \pm 0 \cdot 6$
Factor VII and X (%)	$100 \pm 1 \cdot 1$	$98 \cdot 5 \pm 0 \cdot 9$
Factor V (%)	$100 \pm 1 \cdot 3$	$99 \pm 2 \cdot 1$
Thrombin time (sec)	$15 \pm 2 \cdot 5$	$24 \pm 4 \cdot 5$
Fibrinogen (mg%)	462 ± 173	412 ± 126
Euglobulin lysis time (min)	36 ± 7	61 ± 21
Fibrin monomer complexes (optical density)	$0 \cdot 53 \pm 0 \cdot 17$	$0 \cdot 31 \pm 0 \cdot 11$

Table 2. Coagulation and fibrinolysis in affluent and effluent blood in a rabbit-to-rabbit liver allograft (a mean of 3 experiments)

	Affluent blood	Effluent blood
Platelets in cu mm	$159 \cdot 000 \pm 12 \cdot 200$	$88 \cdot 000 \pm 18 \cdot 200$
Calcium clotting time (sec)	69 ± 14	83 ± 17
Prothrombin time (%)	$100 \pm 0 \cdot 7$	$89 \pm 1 \cdot 5$
Factor VII and X (%)	$100 \pm 1 \cdot 0$	$96 \pm 1 \cdot 6$
Factor V (%)	$100 \pm 0 \cdot 5$	$93 \pm 1 \cdot 2$
Thrombin time (sec)	$15 \pm 3 \cdot 0$	$22 \pm 7 \cdot 0$
Fibrinogen (mg%)	398 ± 176	302 ± 66
Euglobulin lysis time (min)	198 ± 33	145 ± 65
Fibrin monomer complexes (optical density)	$0 \cdot 42$	$0 \cdot 37$

Table 3. Coagulation and fibrinolysis in affluent and effluent blood in a dog-to-dog liver alograft (a mean of 3 experiments)

	Affluent blood	Effluent blood
Platelets in cu mm	$281 \cdot 000 \pm 27 \cdot 400$	$183 \cdot 000 \pm 60 \cdot 200$
Calcium clotting time (sec)	59 ± 13	68 ± 19
Prothrombin time (%)	$100 \pm 0 \cdot 9$	$102 \pm 0 \cdot 7$
Factor VII and X (%)	$1000 \pm 1 \cdot 2$	$105 \pm 0 \cdot 6$
Factor V (%)	$100 \pm 1 \cdot 1$	$101 \pm 1 \cdot 0$
Thrombin time (sec)	$14 \pm 2 \cdot 0$	$15 \pm 2 \cdot 2$
Fibrinogen (mg%)	417 ± 70	494 ± 98
Euglobulin lysis time (min)	47 ± 18	$38 \cdot 5 \pm 18$
Fibrin monomer complexes (optical density)	$0 \cdot 69 \pm 0 \cdot 21$	$0 \cdot 69 \pm 0 \cdot 18$

of platelets were retained in the liver. There was also an increase in calcium clotting time and thrombin time, as well as euglobulin lysis time. A small gradient of fibrinogen concentration across the graft of a mean of 50 mg% was noted. There was an evident decrease in fibrin monomer concentrations in the effluent blood.

Table 4. Collective data of affluent and effluent blood gradient of platelets and coagulation factors across the liver xeno- and allografts

	Rabbit-to-dog	Rabbit-to-rabbit	Dog-to-dog
Platelets (%)	88	45	35
Calcium clotting time (sec)	30	20	15
Prothrombin time (%)	12	9	2
Factor VII and X (%)	1·5	4	5
Factor V (%)	1·0	1·0	1·0
Thrombin time (sec)	60	46	7
Fibrinogen (%)	11	24	4
Euglobulin lysis time (min)	69	27	18
Fibrin monomers (%)	58	7	6

Group 2. The data are presented in Table 2. The blood flow did not change during the first 10 minutes. There was a slight increase in calcium clotting time and thrombin time, and decrease in euglobulin lysis time. A fibrinogen concentration gradient of 96 mg% was noted, together with a considerable tetention of platelets within the graft.

Group 3. The results are shown in Table 3. There was no change either in the blood flow through the organ, or in the coagulation and fibrinolysis system. There was, however, evident retention of platelets in the transplanted liver. The data of all three groups are assembled in Table 4.

DISCUSSION

The problem whether immunologically induced coagulopathy is responsible for cessation of blood flow through the hyperacutely rejected allo- and xenograft has been studied by many authors. Its elucidation would be of prime importance in clinical practice. The percentage of kidney allografts lost soon after transplantation due to hyperacute rejection amounts to 5—10%. Due to the same process, xenografts cannot be successfully transplanted. If intravascular coagulation were responsible for sudden ischemia, it would probably be possible to mitigate it by pharmacological means. There is still much controversy whether coagulation

process takes place in the graft itself, and if so, whether it is a primary or secondary phenomenon. Starzl et al., (1970) upon studying a short series of patients with hyperacutely rejected kidneys, found an evident consumption of coagulation factors within the graft. They confirmed these findings in a series of experimental studies in sensitized dogs, in which the highest consumption rate of coagulation factors was found in livers (Simpson et al., 1970). On the other hand, Colman et al., (1969) did not observe consumptive coagulopathy in 2 patients with on-the-table rejected kidneys. In experimental studies, Rosenberg et al. (1969) observed in pig kidneys transplanted to dogs trapping of recipient platelets, consumption of fibrinogen with increased concentration of fibrinogen split products in the effluent blood, consumption of factor V and VII, prolongation of prothrombin and thrombin time. Studies at the Denver Center carried out by Giles et al., (1970) gave similar results. However, no or only minimal deposits of fibrin were present in the liver. Parallel measurement of hemagglutinins, lymphoagglutinins, cytotoxins to the donor antigens, as well as complement, revealed considerable decrease of titers in the effluent blood.

All the above listed authors support the view that intraorgan coagulation is initiated by the immune reaction between the recipient circulating preformed antibodies and C', and donor cell antigens. This may bring about adhesion of platelets and leukocytes, release of platelet factors and of vasoactive substances, with all its sequelae. Platelet aggregates together with fibrin strands plug the microvasculature. It seems worthwhile to quote here Hutchinson et al., (1968), who found considerable platelet trapping in liver allografts immediately after revascularization, not followed by any evident changes in blood flow. Winch et al. (1972) perfusing pig livers with human blood also observed major blood flow disturbances despite retention of 90% of platelets in the liver. The question whether the immunological damage to the capillary wall, or aggregation of platelets and leukocytes with occlusion of the microvasculature, or intravascular coagulation, or all three factors together, are responsible for transplant ischemia remains open.

In our studies on a model of rabbit-to-dog liver xenografts, no consumptive intra-organ coagulopathy was detected. There was a minimal retention of fibrinogen within the graft. Calcium clotting time and thrombin time were slightly protracted, but this might be due to the residual hepatin, as the graft was washed out prior to transplantation with heparinized saline solution. Blood flow stopped completely at 5 to 7 minutes after restablishment of perfusion. Oddly enough, in the two control groups with liver allografts similar changes as in xenograft group, with platelet retention and prolongation of calcium clotting time and thrombin time, were ob-

served. Platelet retention in an allogeneic system may, at least partially, be due to mechanical injury to the vascular endothelium during elution procedure and protracted ischemia. A normal blood flow was, sustained for the first hour of the experiment. Our findings seem to negate the hypothesis that · enzymatically induced coagulation is responsible for cessation of blood flow in the xenograft. The observation of a striking decrease of soluble fibrin monomers in the effluent blood is noteworthy. It is known (Niewiarowski etal., 1968) that released from aggregated platelets factor 4 or lysosome protein fraction of polymorphonuclear (Hawiger et al., 1969) can in vivo precipitate soluble complexes of fibrin monomers in a process of paracoagulation.

Our further studies will be devoted to investigating the influence of antithrombotic drugs on the mitigation of hyperacute rejection of the liver xenograft and on the intra-organ coagulation process.

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