The Role of the Platelet in the Obliterative Vascular Transplant Rejection Phenomenon

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The obliterative vascular lesion comprises a significant pathological aspect of allograft rejection (Porter et al, 1963; Porter et al, 1964; Busch et al, 1969). Its presence during this phenomenon has been acknowledged by transplantation investigators including Hume (1968), Porter et al (1963, 1964), Dempster (1964) and others. It can be implicated in the pathophysiology seen during acute rejection, including a decrease in renal blood flow, urine output, urine sodium concentration and an increase in urine osmolarity. Generally, it is a reversible phenomenon during the acute phase, but it also plays a role in chronic rejection where reversal is more difficult.

The aggregation of platelets is implicated in this obliterative vasculitis. Lowenhaupt and Nathan (1969) have demonstrated platelet aggregates in small vessels in the early phase of renal allograft rejection. Others (Porter et al, 1964; Busch et al, 1969) have also reported platelet trapping within the rejecting organ with adherence of these platelets to the intimal surface of small vessels leading to eventual occlusion. The potential for deaggregating platelet collections with drugs such as steroids can reasonably explain the reversibility of this obliterative lesion as seen on serial biopsies during and after the treated acute rejection phase.

In an attempt to prevent or lessen this aggregative process a variety of pharmacologic agents with platelet deaggregating properties was examined. Among the most potent and least toxic of these agents was cyproheptadine (Periactin, Merck, Sharpe & Dohme), a common antihistaminic and antiserotonin. This drug is capable of inhibiting ADP, thrombin, and connective tissue serotonin induced platelet aggregation (Aledort, 1970).

In order to demonstrate the possible value of this drug's effect on platelets during the rejection crisis, several experimental protocols were established. In addition, the drug was used in nine human cadaveric renal transplants.

EXPERIMENTAL PLAN

The following groups were studied:

1. Canine renal allografts
2. Canine renal allografts in hyperimmunised dogs
3. Feline-canine renal xenografts
4. Human cadaveric renal allografts

Canine renal allografts

Renal allografts were performed in anaesthetised, bilaterally nephrectomised mongrel dogs by standard operative techniques with the kidney placed in the pelvis. Anticoagulants were not used during grafting. Recipients in the experimental group were placed on 1.2 mg/kg/day of cyproheptadine beginning 2 days prior to grafting, with an additional intravenous dose of 1.5 mg/kg on the day of surgery. Controls received no therapy. Serial BUNs, serum creatinines and urine outputs were used to follow renal function. When rejection was evident, the kidneys were removed. At the time of transplant, and just prior to kidney removal renal arterial and venous platelet counts were performed.

Hyperimmunised renal allografts

Recipient dogs were brought to a white graft state by repeated donor specific skin grafts and/or inoculation with donor specific spleen cells. A few days after a white graft was achieved, a donor specific renal allograft was placed in the pelvis. The animals received cyproheptadine 1.2 mg/kg/day 2 days before surgery, 0.5 mg/kg/hour IV during surgery, 10 mg IM 3 hourly after surgery. At the time of grafting, and just prior to graft removal at the time of rejection, renal arterial and venous platelet counts were done.

Feline-canine renal xenografts

Cat kidneys were removed with their vessels and ureters and placed in the pelvis of anaesthetised dogs by standard vascular operative techniques. Ureterovesical continuity was not re-established. Cyproheptadine was administered to the experimental group 2 days prior to grafting (1.2 mg/kg/day and 1.5 mg/kg IV just prior to grafting). Serial renal arterial and venous platelet counts were done every few minutes and observations were made on renal blood flow and urine output.

Human renal cadaveric allografts

Retrospective and prospective leucocyte matching was performed in most cases. In general, the cyproheptadine treated groups and control groups had similar matching grades. Nine recipients were given one oral dose (4-8 mg) of cyproheptadine just prior to operation; fourteen recipients did not receive

the drug. Postoperatively, the treated group was maintained on 32 mg/day (16 mg/day in children) of cyproheptadine orally in divided doses.

All patients were maintained on post transplant prednisone (high doses – 200 mg/day for 48 hours, then 30-40 mg/day until rejection was evident, then raised again) and Imuran (1-3 mg/kg/day) and local irradiation (150R) to the graft on days 1, 3, 5, 7 post transplant with an additional 1-2 doses at the time of rejection. No other form of anti-rejection therapy was administered. Patients were observed for the onset of rejection by the usual criteria. In most cases, percutaneous transplant biopsies were performed at various times during the post transplant course.

RESULTS

Canine renal allografts

The following chart summarises the findings in this group:

	Untreated	Cyproheptadine treated
Number of dogs	10	10
Kidney survival (days)	5-6	8-14
Platelet count at initial grafting average (range)	A-250,000 (360-180) V-220,000 (330-120)	A-270,000 (320-210) V-250,000 (280-150
Platelet count at re-exploration average (range)	A-200,000 (230-120) V- 65,000 (170-20)	A-250,000 (320-200) V-200,000 (280-140)

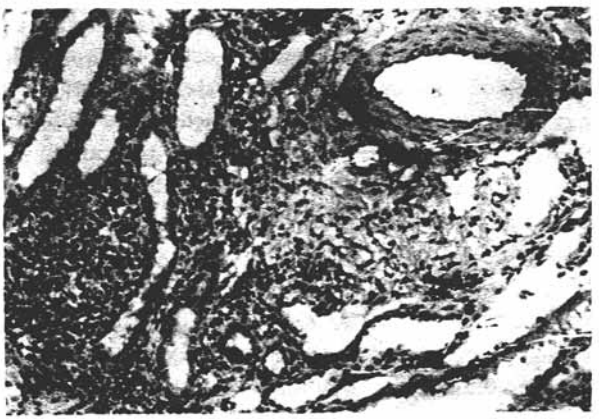

Figure 1. Cyproheptadine treated canine renal allograft. On day 15 one acute rejection. BUN over 100 mg/100 ml. Minimal vascular reaction

The histological picture of rejection in the untreated group was typical of unmodified recipients; that is, a mononuclear cell infiltrate most marked around blood vessels. The endothelial cell lining of small arterioles appeared swollen and many vessels were plugged with platelets and fibrin debris.

In the treated group there was minimal reduction in the calibre of vessel lumina and platelet plugging was not evident. Mononuclear cell infiltration was present (Figure 1).

Hyperimmunised canine renal allografts

The following chart summarises the findings in this group:

	Untreated	Cyproheptadine treated
Number of dogs	2	2
Platelet count at renal grafting average (range)	A-320,000 (440-280) V- 80,000 (90-30)	A-270,000 (330-200) V-250,000 (250-180)
Platelet count at re-exploration 24 hours after transplant average (range)	A-210,000 (260-180) V- 0 (graft necrotic)	A-200,000 (240-150) V- 65,000 (80-40)
Blood flow at completion of grafting	Absent	Present
Urinary output at completion of grafting	Absent	Present

Feline-canine renal xenografts

The following chart summarises the findings in this group:

	Untreated	Cyproheptadine treated
Number of animals	8	13
Platelet count at 6' average (range)	A-300,000 (370-280) V- 90,000 (110-70)	A-310,000 (440-180) V-250,000 (360-160)
Platelet count at 8' average (range)	A-250,000 (350-180) V- 60,000 (0-80,000)	A-300,000 (400-180) V-240,000 (340-160)
Venous platelet count at 60', 90', 180' average (range)	60' - 0 (no flow) 90' - 0 (no flow) 180' - 0 (no flow)	60' - 230,000 (250-160) 90' - 210,000 (250-150) 180' - 200,000 (250-170)
Urinary output	Absent	Present (25% of animals)
Duration of renal blood	8-10	180-420

The histological findings on grafts in the untreated animals excised following cessation of blood flow revealed the arterioles to be plugged with platelets (Figure 2). A scant infiltrate of primarily polymorphonuclear cells

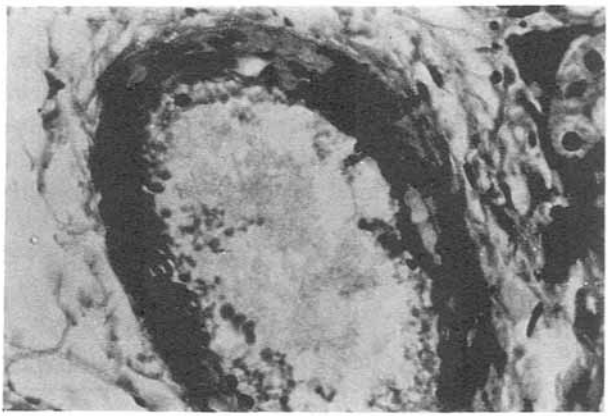

Figure 2. Non-cyproheptadine xenograft 4 minutes after release of arterial clamp. Platelet thrombus forming

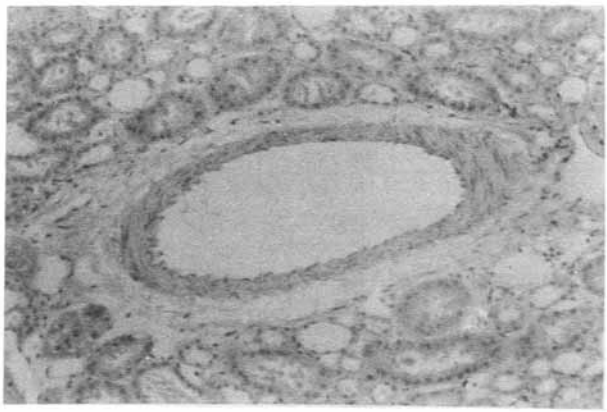

Figure 3. Cyproheptadine treated xenograft 120 minutes after release of arterial clamp. Minimal vascular reaction

was present with numerous areas of haemorrhage into the peritubular spaces.

The cyproheptadine treated animals biopsied at approximately one hour or later revealed minimal platelet clumping in arterioles and glomeruli with numerous areas of interstitial haemorrhage (Figure 3).

Human cadaveric renal transplants

The most significant difference noted was that all the patients (14) who were not treated with cyproheptadine had their first rejection crisis within five to twenty-one days with a mean of fourteen days while the following chart summarises the findings in the cyproheptadine treated group:

Patient	Donor	Match	Rejection Day	Comment
SV	CAD	C	70	Drug stopped on 59th day
EB	CAD		46	
BD	CAD		441	No rejection yet
GS	CAD	C	234	Convulsion day 34 Chronic rejection
ES	CAD	B	101	No rejection Died, sepsis
EA	CAD	D	91	Chronic rejection
JC	CAD	C	188	No rejection Died, perforated DU
LC	CAD	C	57	
LB	CAD	C	81	
	Average 140 days			

The histological findings in the treated group show a definite decrease in vascular lesions even when interstitial evidence of rejection is present (Figure 4, Figure 5).

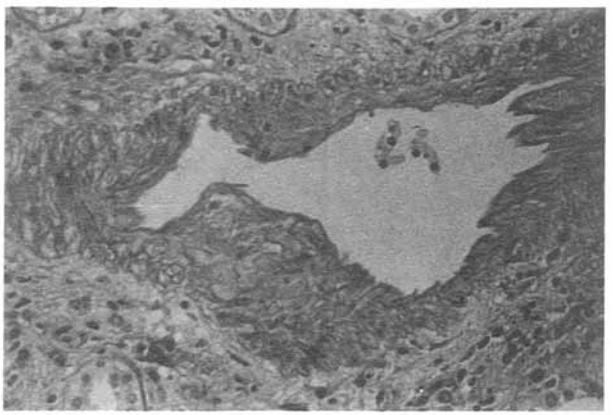

Figure 4. Cyproheptadine treated human allograft day 120. Chronic rejection. Minimal vascular reaction

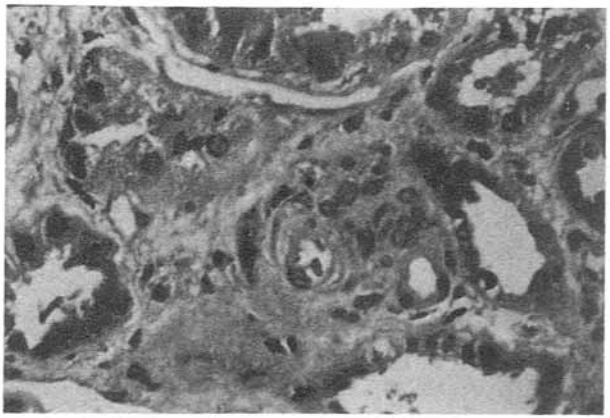

Figure 5. Cyproheptadine treated human renal allograft. Minimal vascular reaction

DISCUSSION

It has been demonstrated in dogs (Porter et al, 1964; Dempster, 1964) and in humans (Porter et al, 1963) that the transplant vasculature is a prime target of allograft antibodies. Endothelial damage, interstitial oedema and round cell infiltration and occlusion of vessels with a decreased renal blood flow are regular features of acute rejection.

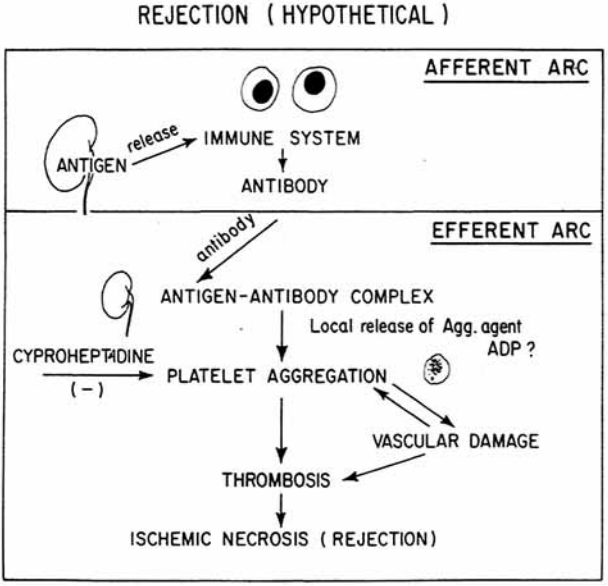

Figure 6. Hypothetical scheme of the role of platelet aggregation in the vascular aspect of rejection

It is currently believed that primary haemostasis occurs at the site of this endothelial damage by the aggregation of platelets (Aledort, 1970). It has also been adequately demonstrated that antigen-antibody complexes themselves produce platelet aggregation and also produce the secondary release of aggregating substances from the platelet (Movat et al, 1965).

The experimental data derived supports the evidence demonstrating the importance of platelet aggregation in the acute rejection process. The mechanisms by which platelets partake in this process are conjectural, but the following diagram can possibly help clarify the process (Figure 6).

The use of a potent platelet deaggregator with the properties of preventing serotonin and ADP induced aggregation has significantly altered platelet trapping in both xenografts and allografts. Other investigators have used deaggregating agents such as phenylbutazone with similar effects (Mathew et al, 1967). Cyproheptadine has the major advantage over heparin and other deaggregating agents of a combination of high potency and low toxicity.

With the use of this agent, xenograft survival has been significantly prolonged. Canine allograft survival has been prolonged slightly, but more importantly renal biopsies suggested a significant reduction in vascular lesions. That rejection occurred eventually is not surprising; it would be too much to expect that a drug without immunosuppressive activity could prevent the nonvascular components of rejection. We have had similar experiences with the use of heparin in canine renal allografts.

The administration of the agent in the human series on concomittant immunosuppressive therapy altered the rejection pattern quite remarkably. The onset of rejection was delayed in all cases and 3 of 9 cases have not experienced one to date. Considering the fact that these are grafts from cadaveric donors, this represents a significant alteration in the transplant course when compared to other series on similar anti-rejection regimens as well as our own non-cyproheptadine series.

It is too early to determine the effects of the drug on ultimate graft survival; three of nine cases eventually lost their kidneys to chronic rejection.

CONCLUSIONS

Platelet aggregation with small vessel occlusion plays an important role in allograft rejection. With the use of a potent, safe platelet deaggregating agent, the acute rejection course can be significantly altered. The drug appears to have its effect on the efferent arc of the rejection cycle and whether it significantly affects ultimate graft survival awaits further study.

REFERENCES

Aledort, L. (1970) 'The Circulating Platelet'. Chapter 5. Academic Press, London

Busch, G. J., Braun, W. E., Carpenter, C. B., Corson, J. M., Galvanek, E. R., Reynolds, E. S., Merrill, J. P. and Dammin, G. J. (1969) Transplantation Proceedings, 1, 267

Dempster, W. J. (1964) Urologia, 31, 3

Hume, D. M. (1968) 'Human Transplantation' (Ed) J. Dausset and F. T. Rapaport. Page 110. Grune and Stratton, New York

Lowenhaupt, R. and Nathan, P. (1969) Transplantation Proceedings, 1, 267

Mathew, T. H., Kincaid-Smith, P., Marshall, V. C. and Eremin, J. (1967) First International Congress of Transplantation Society, 1, 256

Movat, H. Z., Mustard, J. E., Taichman, N. S., Uriuhara, T. (1965) Proceedings of the Society for Experimental Biology and Medicine, 120, 232

Porter, K. A., Calne, R. Y., Zukowski, C. F. (1964) Laboratory Investigation, 13, 809

Porter, K. A., Thompson, W. B., Owen, K., Kenyon, J. R., Mowbray, J. F. and Peart, W . S. (1963) British Medical Journal, 2, 639