INTENSIVE PLASMA EXCHANGE, COMPLEMENT DEPENDENT MICROCYTOTOXICITY AND RENAL TRANSPLANT REJECTION

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Summary

Intensive plasma exchange (IPE) was used to treat 13 rejection episodes in eight renal transplant recipients with biopsy evidence of humoral rejection. Prior to IPE, each patient had several rejection episodes treated with high dose steroids. The IPE-treated rejections had not responded to conventional anti-rejection therapy and all patients appeared likely to lose their grafts. IPE reversed 7 of the 13 rejections (5 of 8 patients responded). Two of the 8 grafts continue to have adequate function 6 and 8 months after IPE. IPE temporarily reverses rejection but may not increase long term graft survival.

Introduction

Uncontrolled rejection is the main cause of renal allograft loss. In non-sensitised recipients, acute early rejections are characterised by cell-mediated graft injury. During subsequent rejections, however, humoral injury plays a variable, but usually, a progressively more important role. The presence of humoral rejection is indicated by narrowing of vessels, alteration in glomerular architecture, and the presence of immunoglobulin, complement and fibrin in vessels.

The presence of immunoglobulins in the vessel wall suggests that antibody directed against graft antigens is responsible, at least in part, for the tissue injury. Antibodies specific for donor HLA antigens have been eluted from grafts with histological evidence of humoral rejection. Circulating anti-HLA antibodies have been found in patients with chronic humoral rejection. It must be emphasised, however, that humoral rejection can occur in the absence of detectable circulating antibodies and in grafts which do not have immunoglobulin in the vessel wall. Thus, other mediators of tissue injury may be important.

High dose steroid therapy for humoral rejection is often ineffective. Patients with ongoing episodes of humoral rejection who receive repeated courses of high dose steroids, are not only likely to reject their graft, but are also at risk from the complications of the steroid therapy. Therapy which is more effective and less toxic is required.
Recent reports indicate that intensive plasma exchange (IPE) appeared to modify favourably the course of patients with Goodpasture's Syndrome and rapidly progressive glomerulonephritis. In the presence of adequate immunosuppression IPE was safe, without significant side effects, and led to long term remission in some cases. The mechanism whereby IPE exerts its effect is not clear but removal of circulating antibody, immune complexes or other inflammatory mediators are the most likely possibilities. Since humoral rejection may be mediated by similar circulating factors, we studied the effect of IPE on graft recipients with histological evidence of humoral rejection. Since IPE may also remove circulating immunosuppressive factors and thus accelerate the rejection process, we restricted IPE therapy to those patients with uncontrolled rejection who appeared likely to lose their grafts. Prior to IPE, all patients had several rejection episodes which responded to high dose steroids. IPE was used to treat a further rejection episode unresponsive to anti-rejection therapy and in our experience, likely to have led to imminent graft loss.

Materials and Methods

Of the 8 patients treated with IPE, 7 patients had received a first cadaveric graft and one a second. There were no 3 or 4 HLA antigen matches. Six of the 8 had detectable circulating cytotoxic antibody prior to transplantation. Chronic glomerulonephritis (5 patients), polycystic renal disease (1 patient), reflux (1 patient), and diabetic nephropathy (1 patient), were the underlying causes of the end stage renal failure.

Thirteen rejections were treated with IPE (5 patients were treated twice). Prior to IPE, all patients had several reversible rejection episodes treated with high dose steroids. The mean number of rejections prior to the IPE treated rejection was 4 (range 2–5). Just prior to IPE, 12 of the 13 rejections treated with IPE had failed to respond to high dose steroids (10 rejections) or antilymphocyte globulin (2 rejections). The rejection process was well established in most patients prior to IPE since the mean serum creatinine at the start of IPE was 5.8 mg/100 ml. Before, during, and after IPE, all patients were maintained on baseline azathioprine and steroids. Four of the 13 rejections received additional anti-rejection therapy during IPE. High dose steroids were given in one and antilymphocyte globulin was given in 3.

A biopsy was done prior to IPE in all patients. Each patient had vascular and glomerular changes compatible with humoral rejection as previously described. All 8 patients had positive immunofluorescence findings in vessels. Immunoglobulin and complement were present in 6 and fibrin in 2.

Cytotoxic antibody was measured in the serum of patients using the complement dependent microcytotoxicity technique. Targets were cell panels from 20 unrelated normals representing most HLA-A, B, C specificities including those of each donor. Cytotoxic antibody was measured before, during, and after 12 of the 13 IPE treated rejections.

IPE was performed once a day using a Haemonetics-30 cell-separator. The mean IPE treatment days per rejection was 6. The minimum number of treatment days was 3 (1 rejection) and the maximum 10 (1 rejection). On each day
of IPE therapy, 4 litres of plasma were removed and replaced with albumin in saline and one unit of fresh frozen plasma.

Results

Seven of the 13 rejection episodes responded to IPE in terms of a fall in the serum creatinine towards baseline after IPE therapy. In the rejections that responded, the mean baseline serum creatinine prior to the onset of the rejection was 3.0 mg/100 ml, peaked at a mean of 8.0 mg/100 ml and after IPE, fell to a mean of 3.3 mg/100 ml. In 6 of the 13, the serum creatinine did not fall. In terms of long term graft survival, 6 of 8 grafts were eventually rejected. Of these 6, one graft maintained adequate function for 9 months after IPE, 3 for approximately 2 months after IPE, and the other 2 were rejected within the month following IPE therapy. Two grafts still maintain adequate function 6 and 8 months post-IPE.

The details of a rejection which appeared to respond to IPE alone are shown in Figure 1. During the first 90 days post transplant, the patient had 4 rejections treated with high dose steroids. The fifth rejection was treated with IPE alone. A biopsy prior to this showed humoral rejection. Steroids were not used for this rejection because of the likelihood that complications would result. The serum creatinine had been stable for one week prior to this rejection. After IPE, the

![Figure 1. Rejection episode treated with IPE alone. \( P \) = one day of IPE therapy; + = detectable cytotoxic antibody; – = cytotoxic antibody not detectable. Serum creatinine is plotted on semi-log scale to accurately reflect creatinine clearance.](image)
serum creatinine returned to baseline and remained stable for approximately 3 weeks. Cytotoxic antibody was detectable during the IPE treated rejection but not afterwards. This patient had a sixth rejection which followed a 2 week period when azathioprine could not be given because of leukopenia. The sixth rejection did not respond to either high dose steroids or repeat IPE. A transplant nephrectomy was done 6 weeks after the first IPE therapy.

Figure 2 illustrates the course of a patient whose rejection process may have been aggravated by IPE. He had 4 rejections in the first 70 days post transplant. All were treated with high dose steroids. A biopsy done on day 66 showed chronic humoral and cellular rejection. Although the serum creatinine was stable for several days at approximately 3.5 mg/100 ml, IPE was started on day 72 because of the humoral changes found on biopsy. IPE was given for 5 days and stopped on day 76. Prior to and during IPE, the patient could be maintained on only small doses of azathioprine (approximately 25 mg/day) because of leukopenia. Following IPE, the serum creatinine began to rise and high dose steroids were given. Eventually, the rejection process subsided and this patient retained adequate function for 9 months (serum creatinine 3.5 mg/100 ml)
when he lost his graft because of uncontrolled rejection. Cytotoxic antibody was not detected before, during, or after the IPE-treated rejection.

In Figure 3, the course of a patient whose rejection process was resistant to steroids but responsive to IPE is illustrated. During the first 119 days post transplant, the patient had 4 rejections, each treated with high dose steroids. A biopsy during this time revealed humoral rejection. The serum creatinine had been stable for 7 days at approximately 2.5 mg/100 ml prior to the fifth rejection. It then started to rise sharply and continued to rise after high dose steroids, although the rate of change had slowed. Nine days after the first steroid bolus, IPE was started. After the fourth day of IPE, the serum creatinine began to fall and stabilised at 3.5 mg/100 ml. Cytotoxic antibody was detectable during the rejection and prior to IPE but not after IPE. This patient has retained adequate graft function 8 months after IPE.

Of the 6 rejections that did not respond to IPE, the following observations were made. Cytotoxic antibody was not detected during rejection in four. In one rejection, cytotoxic antibody was detectable during rejection, but was no longer detectable after IPE. In one rejection cytotoxic antibody was detectable during rejection and after IPE. In four, the serum creatinine had been increasing prior to IPE and continued to rise after IPE. In the other two, the serum creatinine was stable but
elevated prior to IPE and IPE was instituted only because of histological evidence of rejection. In both instances, the serum creatinine increased following IPE. Also, in both, the dose of azathioprine was less than 25 mg/day during and after IPE therapy.

Seven rejection episodes responded to IPE. In all seven, the serum creatinine had been increasing prior to IPE, peaked during IPE, and fell towards baseline after IPE. Cytotoxic antibody was measured in 6 of the 7. In 5 of the 6, cytotoxic antibody was detectable during the rejection but not after IPE. Of the 7 that responded, 5 were the first IPE-treated rejections. Two patients responded to IPE on two occasions. The baseline immunosuppression given during five of these rejections was azathioprine ≥ 100 mg/day and prednisone ≥ 25 mg/day.

No significant side effects resulted from IPE. Two patients experienced mild symptoms of hypocalcaemia and did not require therapy. There was a decrease in the levels of the third component of complement and in gammaglobulins in all patients. No bleeding abnormalities were noted although all patients had low fibrinogen levels and elevated prothrombin times. No significant infections occurred after IPE.

Conclusions

IPE appears to be a safe form of therapy for humoral rejection in transplant recipients receiving adequate baseline immunosuppression. It is potentially harmful when patients cannot be given sufficient immunosuppression since it may remove protective factors and possibly lead to increased antibody production. In this series, all those without detectable cytotoxic antibody during the IPE-treated rejection did not have detectable antibody after IPE. In the two instances where the serum creatinine increased after IPE, two factors must be considered. The first is the fact that in both instances, the daily dose of azathioprine was low prior to and during IPE treatment. The increase in the serum creatinine following IPE may have occurred as the result of inadequate amounts of azathioprine despite the IPE therapy. A second possibility is that IPE disrupts the balance between factors cytotoxic to the graft and protective to the graft, and should not be used when the serum creatinine is stable.

IPE may be more effective if initiated early, as the serum creatinine is rising, and if cytotoxic antibody is detectable. IPE appears temporarily to reverse rejection (5 of 8 patients responded) but may not increase long term graft survival. However, two patients in whom graft loss appeared imminent, have functioning grafts (serum creatinine 3.5 mg/100 ml and 1.2 mg/100 ml) 6 and 8 months after IPE.

The possible role of IPE in the management of transplant recipients cannot yet be fully defined. IPE may be a less toxic but effective alternative therapy to steroids in patients who have had repeated rejections treated with high dose steroids. It may also have a role as an adjunct to steroid therapy for early rejections with predominantly humoral mechanisms, since it may effectively remove circulating factors toxic to the graft.
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References

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Open Discussion

LEGRAIN (Paris) I want to ask about this problem you call ‘unresponsiveness’. It was quite clear that some patients had received high doses of prednisone for at least five days or a week. But was it so for all the seven cases who responded or have you included a few cases which had not responded after only two or three days?

CARDELLA On the sixth day after the first course of steroids we accept that there is steroid-resistant rejection and then institute IPE.

THAYSEN (Copenhagen) Did you try IPE for early rejection episodes when you had antibody present? As far as I could make out, you treated rejections occurring from 70 to 120 days after grafting but not early ones.

CARDELLA In fact we had two treated within the first month, one positive for antibody and one negative. They both responded initially, but on repeat IPE they did not respond. So there are only two examples. We had not treated the first or the second rejection alone with just IPE. They have always received conventional corticosteroid therapy.

MUIR (London) We heard today of the possible benefit of blood transfusion
before transplantation. Is it conceivable that the plasma protein fraction you are putting into the patient is what you are treating them with?

CARDELLA Yes, it is. We have not controlled for that variable and I think that that has to be done in the future studies.

HABERAL (Ankara) Can you tell us how much steroid you give?

CARDELLA Our routine is to give 10 mg/kg for at least three days and taper after that.