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HUMAN ORGAN TRANSPLANTATION UNDER CYCLOSPORIN A AND STEROIDS*

Thomas E. Starzl, Shunzaburo Iwatsuki, Göran B.G. Klintmalm, Kendrick A. Porter and Carlos Fernandez-Bueno

Department of Surgery, University of Pittsburgh Health Sciences Center, Pittsburgh, PA, U.S.A. 15261, and Department of Pathology, Saint Mary's Hospital and Medical School, London W2 England

The inadequacies of conventional immunosuppression with azathioprine and Prednisone have been serious enough to prevent the full exploitation of organ transplantation procedures. The prospect of improving the situation was dependent on new immunosuppression techniques. In 1976, Borel, et al (1,2) described the immunodepressive properties in mice, rats, and guinea pigs of an extract from the fungi Cylindrocarpon lucidum and Trichoderma polysporum. This agent, Cyclosporin A, suppressed cellular and humoral immunity without bone marrow depression or other prohibitive organ toxicity.

Clinical trials were begun in 1978 by Calne, et al (3,4,5) of Cambridge, England with encouraging results. In late 1979, Cyclosporin became available for preliminary testing in the United States (11). We report here our experience with this drug in two institutions, and with three organs (the kidney, liver and heart). Much of this information has been published in detail elsewhere (7,8,9,11).

METHODS OF IMMUNOSUPPRESSION

In the first part of the experience with kidneys and livers, Prednisone was withheld postoperatively until there were manifestations of rejection. When it became obvious that rejection occurred in more than half of the cases, treatment was standardized as follows:

On the day of (or the day before) operation and after, Cyclosporin A was given at a dose of 17.5 mg/kg/day. This was continued daily for two months if possible. Often, this dose was decreased at the end of that time (or before in the event of toxic side effects) to the 10 mg/kg/day range. In adults, Prednisone was started at a dose of 200 mg on the day of operation with decreases of 40 mgm for the next four days. On day five, the dose was reduced from 40 to 20 mg. After this, weaning from the 20 mg/day was on the basis of the clinical course. Deviations from this plan were made if dictated by postoperative complications including the supervention of rejection. Rejections were treated with a one gram bolus of hydrocortisone plus a five day course of high dose oral Prednisone as described above. In small adults or children, steroid therapy was appropriately adjusted.

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For hepatic and cardiac recipients, treatment was made more flexible according to the clinical state of the patients. For extremely ill liver recipients, early Prednisone therapy was scaled down. For heart recipients, the daily decrements from the high of 200 mg/day were by 20 instead of 40 mg/day.

The matches of the recipients with their donors were poor. The number of mismatches at the HLA-A and B loci averaged more than three. At the DR locus, there were no perfect matches in the recipients for whom this information was available. The sera of all recipients were analyzed for antibody content. Many of the graft recipients had warm anti-T or anti-B-lymphocyte antibodies against more than 20% of a lymphocyte panel obtained from healthy volunteers. Two of the liver and one of the heart recipients had transplantation against donor specific T-warm antibodies which cause hyperacute rejection of kidneys. The heart recipient had a stormy course, during which biopsies showed deposition of complement and immunoglobulins in the vessels. Under intensive therapy with hydrocortisone, she recovered and biopsy evidence of humoral rejection receded. The liver recipients were not adversely effected by the preformed antibody state. One A liver was transplanted to a B blood type recipient with no adverse effect.

RESULTS IN COLORADO EXPERIENCE

Renal Transplantation

Sixty-six patients were given 67 renal homografts, including nine recipients who were undergoing retransplantation. No attempt was made to deliberately transfuse the recipients in advance of operation. Many of them were referred from Nephrology centers in which transfusion had been avoided.

Nine patients (13.6%) died from 20 to 335 days after transplantation. Of these nine, five had good or excellent function at the time of death. Two of the deaths were in elderly patients who died of coronary artery disease, and a third patient died of a stroke. These deaths were not related to immunosuppression. Six patients died of opportunistic infections (invariably pulmonary) and these recipients were considered to have been over immunosuppressed.

Follow-up is 11 to 21 months for the 57 patients still living. Fifty (75.8%) of the original 66 patients are free of dialysis.

The serum creatinine concentrations in the 50 patients still bearing kidneys is summarized below. All of these recipients having satisfactory and stable function, but as previously reported by Calne et al (4), a number of patients with clinically good results have slightly abnormal renal function.

TABLE 1. PRESENT GRAFT FUNCTION IN LIFE-SUSTAINING KIDNEYS

SERUM CREATININE	NUMBER OF PATIENTS
$\leq$ 1.5 mg%	9
1.6 - 2.5 mg%	26
2.6 - 3.5 mg%	15

After 4 to 13½ months, Cyclosporin A was stopped in 6 patients. In each instance, nephrotoxicity was suspected. Within 13 days to 10 weeks, two of the recipients rejected their kidneys. The other four have had stable or improved renal function in the ensuing 11, 10, 7 and 4 months.

Twelve kidneys became available for study 14 to 335 days after transplantation. In seven, immunosuppression had been stopped for a significant period previously and six of these grafts showed evidence of rejection. In four it was acute, superimposed in one instance upon chronic rejection, in one it was chronic, and in another acute rejection was just commencing. The seventh kidney was normal 11 days after stopping immunosuppression, except for a few fibrin thrombi in glomerular capillaries and in arterioles.

Five kidneys came from patients who had received continuous immunosuppression. Two of these showed changes of chronic rejection, in one there was mild cellular infiltration, and in the fourth there was no evidence of rejection. The fifth kidney in a patient with oxalosis was crammed with oxalate crystals. There was no rejection.

An unusual feature in three of the seven grafts which showed signs of acute rejection was the presence of eosinophils in the interstitial cellular infiltrate. One graft showed patchy acute tubular necrosis, but there was no morphological evidence of a specific Cyclosporin induced tubular lesion.

The results of kidney survival have been analyzed according to primary cadaveric versus retransplantation (9). After 11 to 21 months, the results are as follows:

Primary Cadaveric Transplantation - Of the 57 kidneys transplanted into 57 recipients, nine were placed in recipients who died (see above) and three more were lost to rejection, two before and one after one year. One kidney failed after 10 months because of recurrent oxalosis. Thus, 44 (77.2%) of the 57 grafts are supporting life. The one year graft survival of 78% is superior to that ever achieved by us with other techniques of immunosuppression, including thoracic duct drainage (in combination with azathioprine and Prednisone).

Cadaveric Retransplantation - Of ten kidneys transplanted into nine recipients, 6 (60%) are functioning after 16½, 16, 14, 13, 12 and 10 months. Three organs were lost to rejection. The fourth was removed because of complete ureteral necrosis, after which retransplantation was carried out successfully.

Liver Transplantation

Between March and September, 1980, 14 patients, ages 8 to 41 years were accepted for the pilot trial of liver transplantation under Cyclosporin A. Two patients who were scheduled to be treated with Cyclosporin A plus Prednisone died during operation. One bled to death from a portal vein laceration and the other received a homograft too large to permit the abdomen to be closed. Thus, only 12 patients were treated with immunosuppression.

The general techniques of liver replacement were as previously described (6,10). Seven of the 14 livers were removed in cities other than Denver (75 to 2000 miles away). All 14 livers were

preserved with Collins solution. Ischemia times were 1½ to 10½ hours. Biliary tract reconstruction was by duct to duct, gall bladder to jejunal Roux limb, and common duct to Roux limb anastomosis, in that order of frequency. Two patients required secondary operations to relieve bile duct obstruction.

Of the 12 patients who survived operation, three had chronic aggressive hepatitis and three had Budd-Chiari Syndrome. There was one example each of primary biliary cirrhosis, sclerosing cholangitis (associated with cholangiocarcinoma), hepatoma, Byler's disease, and intrahepatic atresia. The two patients who died during operation had sclerosing cholangitis and secondary biliary cirrhosis (previous gun shot wound to the hepatic hilum).

Among the 12 patients who survived operation and who could be treated with immunosuppression, the only postoperative death was of a child 19 days after transplantation. The hepatic artery of the homograft had thrombosed.

Two patients died late, one at 12 and the other after 15 months. The first death was due to recurrent cholangiocarcinoma which had been in the excised native liver. The second late death was due to recurrent Budd-Chiari Syndrome.

Nine (75%) of the 12 patients are alive after follow-ups of 10 to 17 months. All but one have normal liver functions, and all are living at home.

Seven of the nine survivors are beyond one year. Including the two patients who died after one year, the actuarial one year survival projection of patients who could be treated with Cyclosporin is 11 of 12 (91.8%). Even including the two patients who died intraoperatively, the one year survival is projected at 78.6%. These results are superior to any in our past experience (6,8,10).

Many liver biopsies were obtained, invariably because of postoperative liver function abnormalities. These biopsies revealed a variety of findings of which the most common was acute cellular rejection. Rejection was always responsive to increased steroid therapy.

RESULTS IN PITTSBURGH EXPERIENCE

Further experience with Cyclosporin A has been acquired at the University of Pittsburgh in renal, hepatic, and cardiac recipients for whom follow-ups are available of two weeks to 4½ months.

TABLE 2:

	NUMBER	ORGANS LOST	ORGANS FUNCTIONING	DEATHS
Primary Cadaveric Kidney	16	1	14	0
Cadaveric Kidney Retransplantation	15	1	11	0
Livers	10	4	6	4
Hearts	4	0	4	0

Thus far, the only loss of a primary or secondary kidney graft was from technical error (one renal vein and one renal artery thrombosis). Four of the kidney grafts are viable by radionuclide flow scan, but not functioning well.

The first four liver recipients died. Common to all the failures was poor initial function of the grafts which was thought to be due to ischemic injury during procurement and preservation. The next six recipients have done well.

The four heart recipients are well, including the one whose serum had donor specific preformed T-warm cytotoxic antibodies.

MORBIDITY FROM IMMUNOSUPPRESSION

The infections in the first renal recipients were due to over-immunosuppression with steroids in an imprudent effort to salvage kidneys. The pneumonidites were caused by Pneumocystis carinii, nocardia, or were of undetermined etiology. One patient had a lung abscess which was treated with left lower lobectomy after immunosuppression was stopped. A number of patients had Herpes simplex at some time. There were no examples of Herpes Zoster. Miscellaneous infections included histoplasmosis, and amebic colitis.

The well being of patients treated with Cyclosporin A was remarkable, mainly because of the low doses of Prednisone which usually were in effect by the end of five days. Hirsutism, gum hyperplasia, flushing and paresthesias after drug ingestion, and tremors were minor annoyances.

One renal recipient died of pulmonary emboli and pneumocystis pneumonia 110 days after transplantation. At autopsy, an incidental finding was small nodular deposits of a B-cell immunoblastic sarcoma in the retroperitoneal and para-aortic lymph nodes, in the spleen, in the liver and in the heart. Much of the tumor was necrotic. Immunoperoxidase techniques revealed that the tumor was monoclonal, many of the cells producing mu heavy chains and kappa light chains. The origin of the tumor could not be determined because host and donor were of the same sex.

Another renal recipient had a mid-small bowel perforation 157 days after transplantation. She had a segmental intestinal resection and remains well more than a year later. Her Cyclosporin dose was reduced from 15.8 to 5.9 mg/kg/day. In the base of the perforated ulcer were nests of lymphoid cells. Determination of intracellular immunoglobulins by immunoperoxidase techniques indicated that these cells were polyclonal, 95% of the cells producing alpha and 5% gamma heavy chains. The kappa light chain to lambda light chain ratio was 6:1. The cells showed female chromatin markers, i.e., they were of host orgin because the donor was male. The eventual diagnosis in the concensus opinion of a number of pathologists was that the lesion was a lymphoproliferative reaction, not a lymphoma.

In both patients, the stored sera before and after renal transplantation were examined for antibodies against Epstein-Barr virus. In both cases the antivirus capsidantigen titer rose significantly after renal transplantation. In the second patient, this was the result of a primary infection. The patient with a lymphoma had antibody before transplantation suggesting reactivation of EB virus.

Neither patient showed increased antibody production to cytomegalovirus or Herpes simplex virus (9).

DISCUSSION

Thus far, the clinical trials with Cyclosporin A have escaped the disillusionment of other promising developments in immunosuppression. However, they have raised questions about the optimal use of this agent, which will have to be taken into consideration by those planning randomized trials. The most persistent of the questions has been whether to use Cyclosporin A as the sole immunosuppressive therapy as practiced by Calne et al (3,4,5), or to combine it from the beginning with Prednisone as we have recommended (7,8,9,11). Because the survival of heart and liver recipients is synonymous with graft survival, the omission of steroid therapy in such patients would be hard to justify. In renal recipients whose viability can be maintained by hemodialysis, studies using Cyclosporin A alone are still justified.

Successful use of Cyclosporin in transplantation of human homografts has depended upon a thorough understanding of the drug's toxicity. Injury to the kidney and liver were monitored in all organ recipients with a view to dose reduction when indicated. The toxic properties of Cyclosporin A were not serious enough to prevent its chronic use. Most of the other side effects of Cyclosporin A have not been serious, including gum hyperplasia, tremor, and regional flushing or vague abdominal discomfort just after drug ingestion.

The most publicized question about Cyclosporin has concerned its potential oncogenicity. It has been known for 15 years that the price of effective conventional immunosuppression is an increased incidence of de novo tumors of which approximately 1/3 are lymphomas. We have seen only one lymphoma in our experience with 123 patients treated with Cyclosporin. To our knowledge no epithelial tumors have been seen in any center. As experience with Cyclosporin A has accumulated worldwide, the specter of this drug being a spectacular tumor producer has receded.

CONCLUSION

Unless some other, as yet unrecognized, side effect emerges to limit the usefulness of Cyclosporin A, this agent should permit significant advances in whole organ transplantation. The early results in our preliminary trials have been better than in any of our previous series of renal, hepatic, and heart transplantations in our institutions, in spite of the inclusion of many recipients who were at higher than average risk. Cyclosporin A will be particularly important in making practical the transplantation of extrarenal organs.

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