CYCLOSPORIN A IN CLINICAL ORGAN GRAFTING

R. Y. CALNE, K. ROLLES, D. J. G. WHITE, S. THIRU*, D. B. EVANS**, R. HENDERSON**, D. L. HAMILTON**, N. BOONE***, P. McMASTER, O. GIBBY*** and ROGER WILLIAMS****

Department of Surgery
University of Cambridge
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FOLLOWING Borel's description of a new immunosuppressive agent, a fungal cyclic peptide, Cyclosporin A (CyA), which had immunosuppressive action 1 a number of experimental papers demonstrated that the agent was a potent inhibitor of rejection of organ allografts in a variety of species 2, 3, 4, 5, 6, 7, 8. Green and Allison 9 found that a short period of treatment with CyA given to rabbits with renal allografts could be followed by prolonged acceptance of the transplant and suggested that CyA might be eliminating clones of lymphocytes reactive against the allograft in question. The mode of action of CyA is not understood but there is agreement by independent workers that the agent is more active against proliferating T-cells than other members of the lymphoid series 10, 11, 12. We have published two interim reports on a pilot study of CyA 13, 14 initially as the only immunosuppressive agent in human recipients of cadaveric organ grafts. This study has now been in progress for two years. Fifty-eight patients have received 65 cadaver organ grafts, 51 kidney grafts, eight segmental vascularised pancreas grafts and six orthotopic liver allografts. The 58 patients range in age from 2 to 59. Nineteen were over 50 years. Two renal allografts were second transplants after the first had failed for technical reasons. The remaining 62 organs were first grafts. All received organs mismatched for A and B locus antigens. In most cases there were two or more mismatches. Details of DR matching are not available. All but one of the 51 recipients with renal allografts had previously been given blood transfusions. Six of the renal allograft recipients also received segmental grafts of pancreas from the same donor. Six patients received orthotopic liver grafts. One of these also had a segmental pancreatic graft. One patient with rapidly progressive retinopathy and severely impaired vision from insulin dependent diabetes received a segmental pancreas allograft alone. Of 51 patients who received renal allografts CyA was given at an initial dose of 25 mg/kg/day in seven cases, 10 mg/kg/day in four cases and 17 mg/kg/day in the remaining forty cases.

The tail and body of the pancreas vascularised from the splenic vessels were transplanted into the right iliac fossa, according to the technique of Gleidman 15.

* Department of Pathology, University of Cambridge
** Dialysis Unit, Addenbrooke's Hospital, Hills Road, Cambridge
*** Department of Biochemistry, University of Cambridge
**** Liver Unit, King's College Hospital, London

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The duct was injected with latex solution based on the technique of Dubernard. In seven cases a small window was cut in the peritoneum and the omentum brought through the window and wrapped around the pancreas. In three patients a distal splenic arteriovenous anastomosis was constructed to increase the flow of blood through the splenic vessels following experimental studies on the blood supply of pancreatic allografts in dogs.

The patients with orthotopic liver allografts received CyA 10 mg/kg/day starting dose. CyA was given initially intramuscularly for the first two or three days in all patients. Subsequently they were changed to an oral preparation.

RESULTS

Renal Grafts

Despite the most careful assessment of a new drug in animals, when it is first used in patients the clinical course cannot be predicted and there may be unexpected dangers. This has been the case with CyA and as certain undesirable characteristics of the drug were observed in man so the protocol was modified. The actuarial survival of functioning renal allografts in the whole series is shown in Fig 1.

The most serious side effect of Cyclosporin A, nephrotoxicity, became apparent shortly after the trial commenced. There was impairment of renal function, oliguria and sometimes anuria, with no light or electron microscopical changes on renal biopsy to explain the toxic effects of the drug. Since a mild cellular infiltrate was common in these biopsies, patients with any changes attributable to an immune reaction were treated with other immunosuppressive agents and
six patients were given Cytimun, a cyclophosphamide derivative, and steroids in addition to CyA. Five of these patients died from infections and one of these was found to have a jejunal lymphoma at post-mortem examination. Four of the first 15 patients had been given prednisolone in addition to CyA for suspected rejection. One of these patients died after four months from septicaemia with pneumonitis and lymphoma in the lung, liver and lymph nodes. In this whole series there were 13 deaths six of these were in the first fourteen cases, all dying of sepsis. It was noted that four patients deliberately hydrated and given mannitol in the perioperative phase had primary diuresis without signs of significant nephrotoxicity from the CyA. It was therefore decided in May 1979 to change the policy of management as follows. All renal allograft recipients would receive 17 mg/kg/day of CyA as an initial starting dose. This would be dropped slowly according to renal function and eventually tailed down to as low a dose as would maintain satisfactory allograft function. Only patients with kidneys that were diuresing at 3 hours would be given CyA. Non-diuresing patients would be given conventional immunosuppression. All patients would be deliberately hydrated and given mannitol during the operation and postoperatively. If secondary anuria occurred a renal biopsy would be performed. If this showed rejection the patient would be given a course of steroids. The dose of steroids and timing of treatment of rejection crises varied initially, but eventually was settled at a maximum of six one gram doses of Solu-Medrone over 14 days. If rejection continued, the kidney would be abandoned or the patient changed to conventional immunosuppressive agents, Imuran and steroids.

The actuarial kidney allograft survival curve of 32 patients receiving kidneys without other organs, who were hydrated and given mannitol and managed

![Figure 2](image-url)
according to the proposal outlined above is shown in Figure 2. There were four failures in these 32 patients, three of whom died:

(1) from a combination of secondary hyperparathyroidism and pneumocystic infection in the lung,

(2) from lung sepsis and disseminated intravascular coagulation, septicaemia and fungal abscesses. This patient had poor renal function and there were hypertensive changes in the donor kidney at the time of transplantation.

(3) from haemorrhage from the inferior epigastric artery following renal biopsy during an episode of impaired renal function, probably due to CyA nephrotoxicity. The renal biopsy was normal.

Another patient in this group of 32 developed purulent bacterial sepsis around the kidney and had an allograft nephrectomy and is now being maintained on dialysis.

Four of the 32 patients have been changed to conventional immunosuppression, between three weeks and five months after grafting. Renal function has varied from normal to good, the highest serum creatinine in patients still receiving CyA being 430 mg/ml and the lowest 60 in a 2 year old child.

Pancreas Grafts

Of the eight patients receiving pancreas allografts two died, one on the third day from overhydration. The kidney had not responded to hydration and mannitol. This patient had a cardiac arrest from fluid overload and both organs were vascularised at the time of death. The second death was from pneumonia after removal of the pancreas allograft, which had been rejected and was bleeding. Subsequently, the kidney was rejected and the patient developed septicaemia. One graft became infarcted due to primary vascular thrombosis, and the pancreas was removed. This patient's kidney graft was rejected and also removed. He had since been retransplanted with another kidney and is being treated with azathioprine and steroids and now has a functioning renal allograft. Five patients have functioning pancreatic allografts and are not requiring insulin or steroids. Three of these patients have kidney allografts, one a liver allograft; the fifth has only a pancreas. It will require study over a number of years in patients with functioning allografts, to determine whether this form of treatment will prevent progression of microangiopathy. The five patients have normoglycaemia and good function of their additional allografts 19. Three of the patients had distal splenic arteriovenous anastomoses constructed. Two of these pancreases are functioning well. The third rejected the allograft and bled from the splenic vein.

Liver allografts

Of the six patients receiving liver allografts using CyA, one had a segmental pancreatic allograft (see above) and is alive and well after nine months. Another patient with a hepatoma has had recurrent hepatoma in the lungs excised. These deposits were present at the time of operation but had not been detected
preoperatively. She is still on CyA with good liver function. Two patients developed renal failure in the early postoperative phase after liver grafting. Both had been on the verge of renal failure prior to liver grafting. Both required dialysis postoperatively. Renal function improved and in one case the immunosuppression was changed to azathioprine and steroids. This patient is alive and well. The other patient had the CyA stopped because of the renal function impairment. She developed progressive jaundice, which on biopsy was found to be due to rejection of the liver, in which the intrahepatic bile duct epithelium was severely damaged. She died from liver failure. Another patient required Solu-Medrol pulses for rejection crises which were not controlled. The CyA was stopped and she now receives Cytimun and prednisolone. This limited experience of CyA in liver grafting shows that a dose of 10 mg/kg has an immunosuppressive effect, but rejection crises have occurred. There has also been nephrotoxicity. We have not observed hepatotoxicity in these patients but in the presence of rejection of the liver, some of the hepatic functional impairment could have been due to CyA.

COMPLICATIONS

Lymphomas

The three lymphomas in this series are the subject of a separate publication 20. Two were found incidentally at postmortem examination in patients dying from sepsis. The third case occurred in a patient who was not hydrated and did not receive mannitol. The allograft was anuric and biopsy after a week showed mild tubular necrosis. No other drug was added and there was diuresis from day 16. Good renal function ensued. Three months after operation he developed a sore throat and in retrospect from an analysis of titres of EB virus he probably had an attack of acute mononucleosis at this time. A month later he developed weight loss and dyspepsia. Endoscopy showed ulceration in the lesser curve of the stomach and duodenum. Biopsy of the duodenal ulcer showed lymphoma. Gastro-duodenal resection was performed in September 1979, five months after his transplant and the CyA was reduced to a dose of 100 mg/day, approximately 1.5 mg/kg. He gained weight, felt well and went back to work. Seven months after resection of the gastro-duodenal lymphoma and reduction of CyA dose his renal function deteriorated and he became hypertensive. Biopsy showed changes of acute cellular rejection, together with some scarring and atrophy of areas of the renal parenchyma. The CyA was stopped and he was given azathioprine and prednisolone and his hypertension was brought under control. His renal function has now improved and there is no clinical evidence of recurrence of the lymphoma. It is well known that effective immunosuppression, particularly with agents that impair T-cell function lead to a high incidence of lymphoma 21 22.

A relationship of lymphoma to viral infection and lack of T-cell control of proliferating B-cells has been suggested 23. A further case similar to our own has been reported in a patient with a renal allograft given CyA and steroids 24. The hazards of lymphoma will remain with effective immunosuppression but in none
of the 32 patients managed according to our revised protocol has lymphoma occurred, so it may be possible to avoid a high incidence of lymphoma if CyA is used correctly.

Mild impairment of liver function tests and fine tremor of the hands were described as common in our previous reports, but with our recent modified protocol of management these have been less frequent and not severe and the incidence of infection has not been high. The increase in growth of fine hair on the face and body does not usually bother patients greatly. The gum hypertrophy can be annoying in some patients but is usually not an important disability. Reactivation of virus infection and the development of de-novo virus infections have occurred as with other immunosuppressive regimes 25.

There is no evidence of permanent structural damage to the kidneys in patients treated with CyA. Some kidneys have had scarring on biopsy but this could well be explained as being due to a combination of hypertension and previous rejection episodes. Since CyA is acutely nephrotoxic it is possible that the drug could aggravate structural changes due to other causes but there is no direct evidence of this. One of the main clinical impressions is the rapid reversal of the nephrotoxic effect when CyA dose is reduced or stopped.

Breast lumps

Two of our patients developed benign breast lumps after CyA treatment. One patient had had previous dysplastic changes in the breast and developed a benign fibroadenoma one year after grafting. Another patient treated only with CyA developed a fibroadenoma which was resected, one year after grafting. Three months later she developed lumps in both breasts and two fibroadenomata were removed from the same breast as previously. The lump in the other breast was not excised. The CyA dose was reduced to 2 mg/kg. Six weeks later renal function rapidly deteriorated and a renal biopsy was performed. This was the first renal biopsy this patient had had and the changes were of severe cellular rejection with no scarring of the kidney, no chronic vascular changes and no acute vasculitis. This patient had been treated with CyA for nearly two years and there was no evidence of structural damage that could be attributed to the drug. The acute rejection following reduction in the dose of CyA had the appearances of an early unmodified cellular reaction. It has responded well to conventional treatment with Imuran and steroids.

DISCUSSION

CyA has also been used in patients with marrow grafts for the treatment of graft-versus-host disease 26. It has been found to be very effective in these patients and none has developed a lymphoma 27. Other centres have started using CyA in pilot studies with varying regimes, but to date there are no reports in the literature. Based on our experience and in an attempt to prevent repeating errors we have made, our conclusions would be as follows:
Cyclosporin A is an extremely powerful immunosuppressive drug in man. We would recommend that the agent be used initially as the sole immunosuppressive drug. In patients receiving renal allografts at a dose of 17 mg/kg/day as a starting dose, reducing the dose at 2 weeks dropping 2 mg/kg every month until a maintenance dose of between 6 and 8 mg/kg/day is reached and staying at around this dose provided that renal function is satisfactory. It is quite clear that if the dose gets below 2 mg/kg/day acute rejection can occur, described in two patients above. We believe that only patients with diuresing renal allografts should be given CyA and if there is secondary anuria, biopsy should be performed. If this shows rejection, up to six doses of Solu-Medrone should be given over a course of two weeks. If these fail to control rejection, the patient should be changed to azathioprine and steroids and CyA stopped. The need for continuing CyA in man or changing to azathioprine and prednisolone argues strongly against the suggestion that the drug acts by clonal deletion. Eight patients have been switched from CyA to azathioprine and steroids with good function in their allografts at present. Although two of our patients are continuing with CyA and long-term prednisolone, we are trying to wean them slowly off the steroid. It is our impression that CyA is better used alone than with long-term steroids. If CyA and a short course of Solu-Medrol does not control rejection, we prefer to change to azathioprine and prednisolone.

Of the patients changed to conventional therapy, two were changed because CyA could not be continued at the high dose, in one case due to recurrent fibroadenomata and the other due to restricted lymphoma. The remainder were changed because of failure to respond to one course of Solu-Medrone.

One liver recipient was changed because of acute renal failure, possibly aggravated by CyA. A further patient, who rejected his kidney and pancreas, following removal of the allografted organs received a second kidney transplant and was treated with azathioprine and steroids with good function in his renal graft.

Our early results with segmental pancreas allografting show that control of diabetes can be achieved with CyA as an immunosuppressant. A two year old child has been successfully transplanted with a kidney and although requiring steroids for rejection, is now receiving only CyA. Previously it has been our policy not to transplant young children because of the side effects of steroids.

Thirteen of our patients have never received steroids and have good allograft function being treated only with CyA. Another 16 are not now receiving steroids or any other immunosuppressive agent, apart from CyA. Out of 58 patients, 29 have not required long-term maintenance with steroids. This is a particularly attractive feature of CyA. CyA being totally insoluble in water and aqueous solvents requires administration in an oily solution. Absorption probably depends on the presence of bile salts, and satisfactory transport mechanisms through the gastro-intestinal wall. Absorption from intramuscular injection may be slow. Impairment of liver function may prevent adequate absorption of the drug and since the liver is probably responsible for most of the excretion of CyA, if the drug is given parenterally liver damage may cause high blood levels of the drug.
A proportion of CyA and its metabolites are excreted in the kidneys, therefore impaired renal function could also result in high CyA blood levels (personal communication Sandoz).

The pharmacokinetics of CyA in man have not been determined to a satisfactory extent because of difficulty measuring the drug. Radioimmunoassay techniques have been technically unsatisfactory; more consistent results are now being obtained (personal communication Sandoz). It may well be that there are marked individual variations in metabolism, absorption and excretion of the agent and titration of blood levels will be the best way of administering the drug. Since such developments in the use of the drug are not yet available, the question arises whether there is sufficient knowledge and experience to justify continuing use of CyA based on empirical observations and pilot studies.

It may well be that there are marked individual variations in metabolism, absorption and excretion of the agent and titration of blood levels will be the best way of administering the drug. Since such developments in the use of the drug are not yet available, the question arises whether there is sufficient knowledge and experience to justify continuing use of CyA based on empirical observations and pilot studies. In the evolution of the protocol of CyA management in our trial, we are now obtaining good results and are able to transplant patients previously thought to be unsuitable, namely those with insulin-dependent diabetes and children. We feel that there is now sufficient information to mount a controlled trial of CyA based on the above protocol, to compare it with conventional immunosuppression using azathioprine and steroids.

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