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Azathioprine-cyclosporin A (CyA) double therapy versus CyA alone after the first rejection episode in kidney-transplanted patients under CyA

A randomized study

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Abstract. A controlled trial was carried out in 78 kidney transplant recipients under cyclosporin A (CyA) monotherapy who had experienced a first rejection episode. Thirty-nine were randomly selected to receive azathioprine (AZA; 2 mg/kg per day) in combination with CyA (group AZA^+), while the others continued to receive CyA alone (group AZA^-). Four of the patients in the study died; three were in group AZA^+ and the cause of their deaths was cardiovascular. Graft survival rates were 97% at 6, 12, and 24 months postrejection in group AZA^+ as compared to 97%, 90%, and 81%, respectively, in group AZA^- ($P < 0.05$ at 12 and 24 months). Significantly more patients were free of rejection with the double therapy than with CyA monotherapy (75% vs 51% at 12 months; $P < 0.05$). In spite of the addition of a second immunosuppressive drug, the CyA dosages given and the CyA trough blood levels maintained were similar in the two groups. Serum creatinine was similar in patients with and without AZA. Infectious complications were also similar in both groups. A significant macrocytosis was the only side effect of AZA therapy. On the whole, these data show the benefit of CyA-AZA double therapy in the prevention of rejection recurrence without exposing patients to either increased risk of infection or serious side effects of AZA. Whether this double therapy should be systematically administered to all recipients or only after a first rejection episode is discussed.

Key words: Cyclosporin A monotherapy in kidney transplantation - Azathioprine in kidney transplantation.

Cyclosporin A (CyA) is unanimously acknowledged as being a major immunosuppressive drug; yet, how it is administered in kidney transplantation, when it is introduced, and which other immunosuppressants it is combined with is still controversial. Merion et al., in their report of the first clinical experience with CyA [7], advocated giving it as a monotherapy, with 41% of their patients receiving CyA alone at 5 years postgrafting. Despite these demonstrative data, a vast majority of transplant groups still administer CyA with some other immunosuppressive drug, most often low-dose steroids, even though this has never been proved to be safer. Randomized trials would be the only reliable ones from which to draw conclusions, but few are available. From those that are [3, 8], it appears that combining a second immunosuppressive treatment with CyA reduces rejection frequency more effectively than CyA monotherapy, but it has the disadvantage of increasing infectious risks.

In our center, CyA has always been used as a monotherapy in the maintenance treatment of kidney recipients. The prevailing attitude has been that CyA alone is probably sufficient unless otherwise indicated by certain clinical events during or after transplantation. Thus, the addition of a second immunosuppressive drug would most likely be considered in order to prevent rejection recurrence in patients having already experienced a first rejection episode.

In the present study, a number of kidney recipients under CyA monotherapy were, after their first rejection episode, randomly selected to receive azathioprine (AZA) together with CyA as an immunosuppressive regimen in order to demonstrate the validity of this strategy and to assess the risks and benefits of the addition of a second drug.

Patients and methods

Of all the recipients of a kidney transplant grafted in our center between July 1982 and December 1987, 78 who presented a first episode of acute rejection were included in this study. It was a first

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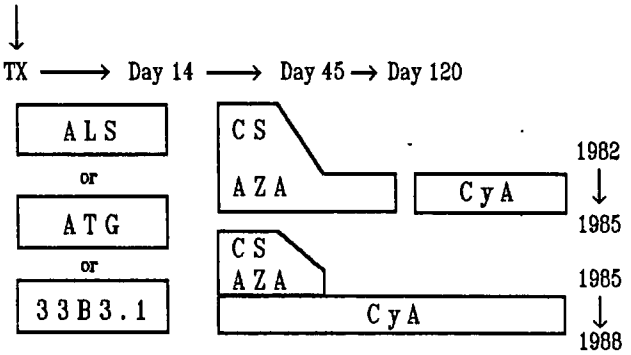

Fig. 1. Description of the different protocols of treatment used in the study. AZA, Azathioprine; CyA, cyclosporin A; CS, corticosteroids; ALS, antilymphocyte globulin; ATG, antithymocyte globulin

transplant for all of the patients. Immunosuppression during the 1st 2 weeks postgrafting consisted of either antilymphocyte/antithymocyte globulins (ALS-ATG, Institut Merieux, Lyon, France) [4] or a monoclonal antibody directed against the IL2 receptor (33B3.1) [11] combined with corticosteroids (CS) and AZA, as previously described [4]. From 1982 to 1985, CyA was introduced during the 3rd month, and after 1985 at day 14 as a monotherapy, after CS and AZA had been tapered off over a period of 4-6 weeks (Fig. 1). The diagnosis of rejection was made on the basis of clinical evidence, but whenever there were doubts, a transplant biopsy was performed. Rejection episodes under CyA were treated by CS boluses (5 mg/kg per day × 2 days, 4 mg/kg per day, 3 mg/kg per day, 2 mg/kg per day). Patients were then randomly selected to receive (or not receive) AZA (2 mg/kg per day) in combination with CyA (groups AZA^+ and AZA^-, respectively). AZA was also introduced in group AZA^- when more than two rejection episodes occurred. CyA treatment was adjusted according to CyA trough blood levels as measured by RIA (accepted values 300-700 ng/ml). Statistical analysis included Student's *t*-test for comparison of mean values and Yates' corrected chi-square test.

Thirty-nine patients (24 male, 15 female) were randomly selected for group AZA^+ and 39 others (30 male, 9 female) for group AZA^-. All were cadaveric graft recipients except for 3 in group AZA^+ who had a semi-identical living related donor kidney. There were no serious imbalances of selection between the two groups, although the proportion of hyperimmunized patients in group AZA^+ was higher than in group AZA^- (20% vs 5%). The initial post-transplantation treatment consisted of polyclonal globulins for 61% of group AZA^- patients and of 33B3.1 for 39% of these patients, compared to 76% and 24%, respectively, of those in group AZA^+. The rejection episode after which randomization started occurred at different times postgrafting; the mean was 16 weeks (range 6 days to 124 weeks) in group AZA^- and 23 weeks (range 5 days to 145 weeks) in group AZA^+. The severity of rejection episodes did not differ in terms of serum creatinine increase or patient response to treatment. The 78 patients had a follow-up of at least 6 months; for similar numbers of patients in the two groups, follow-up extended to 12 and 24 months (28/39 and 13/39, respectively, in group AZA^-; 27/39 and 10/39, respectively, in group AZA^+).

Table 1. Recurrence of rejection. *N*. Total number of patients observed at each point

Time after the first rejection (months)	Patients without recurrence of rejection			
	AZA−	*n*	AZA+	*n*
+ 3	74%	39	81%	39
+ 6	62%	25	78%	29
+12	51%	17	74%	19
+24	41%	5	74%	10

* $P<0.05$

Results

Patient survival

One of the 39 patients in group AZA^- died of legionnaires' disease after an Epstein-Barr virus (EBV) infection. In group AZA^+, three deaths occurred; in all cases, the cause was cardiovascular.

Graft survival and rejection recurrence

When patients who died are excluded, actuarial graft survival in group AZA^+ was 97% at 6, 12, and 24 months after randomization, while it was 97%, 90%, and 81%, respectively, in group AZA^- ($P<0.05$ at 24 months). AZA seemed effective in preventing a second rejection episode, as a significantly higher percentage of recipients in group AZA^+ than in group AZA^- were free of recurrence at 6 months (78% vs 62%), 12 months (74% vs 51%; $P<0.05$), and 24 months (74% vs 41%; $P<0.05$) after their first rejection episode (Table 1).

CyA nephrotoxicity

The CyA requirement, and subsequently its possible nephrotoxicity, were assessed in each group, from the mean serum creatinine, the CyA dosage and its blood level (RIA) maintained in each group. Table 2 shows that although serum creatinine was higher in group AZA^+ 3 months after randomization, there was no difference at 12 months. Although not significant, patients in both groups who had more than one rejection episode had a higher serum creatinine than patients who had not rejected (data not shown). CyA dosages were identical in both groups. RIA blood levels of CyA in the two groups were not significantly different at 3 months postrejection and were, in fact, similar at 12 months.

Infections

Twenty-eight percent of the patients in group AZA^- and 20% of those in group AZA^+ presented at least one significant infectious episode. Most of

them in both groups were herpes viruses, cytomegalovirus (CMV) infections, or EBV infections, and these occurred exclusively in patients who were randomized in the 1st 3 months postgrafting. When mild, but recurring, infections that can also reflect a state of chronic immunodepression were excluded, a similar number of patients (66% in group AZA^- vs 69% in group AZA^+) were found to be completely free of infection.

Complications of AZA therapy

No toxic hepatitis related to AZA occurred in the study. White blood cell counts, platelet counts, and hemoglobinemia were similar in patients receiving and not receiving AZA at 3 and 12 months (Table 3). However, mean red blood cell globular volume tended to increase under AZA treatment, and the difference with group AZA^- reached statistical significance ($P<0.05$) at 12 months.

Table 2. Comparison of serum creatinine, CyA dosage, and blood levels (RIA) in patients with and without azathioprine (AZA; mean ± SD) at 3 and 12 months after rejection (P= NS)

	AZA^-	AZA^+
Serum creatinine (μmol/l)		
3 months	176 ± 80	207 ± 83
12 months	194 ± 76	185 ± 68
CyA (mg/kg per day)		
3 months	5.7 ± 1.75	5.7 ± 2.55
12 months	5.6 ± 2.5	5.6 ± 2.45
RIA (ng/ml)		
3 months	535 ± 171	437 ± 189
12 months	416 ± 177	428 ± 140

Table 3. Hematological complications of azathioprine (AZA; mean ± SD) 3 and 12 months after rejection

	AZA^-	AZA^+
White blood cell counts (cells/mm^3)		
3 months	6,380 ± 3,720	6,060 ± 1,600
12 months	5,960 ± 2,740	5,910 ± 1,660
Platelets (cells/mm^3)		
3 months	260,000 ± 54,000	306,000 ± 87,000
12 months	254,000 ± 69,000	263,000 ± 73,000
Hemoglobin (g/100 ml)		
3 months	11.8 ± 2.4	10.6 ± 1.5
12 months	12.2 ± 1.9	11.8 ± 1.5
Mean globular volume		
3 months	88 ± 8.5	90 ± 6.6
12 months*	90 ± 8.1	96 ± 8.7

* $P<0.05$

Discussion

This study was designed to analyze the effect of adding AZA to CyA therapy on the prevention of rejection, on the occurrence of side effects of AZA, and on the occurrence of infectious complications resulting from increased immunodepression. In addition, although the protocol did not imply doing it, attention was paid to a possible decrease in CyA dosages in recipients receiving AZA and, therefore, to a possible reduction in CyA nephrotoxicity.

Indeed, the value of combining AZA with CyA therapy was demonstrated in the prevention of further rejection in kidney recipients having already presented a first episode. Graft survival improved and the frequency of rejection recurrence in patients receiving the double therapy was significantly lower, as early as 6 months after the first rejection episode. These findings are not in complete agreement with other randomized studies [3, 5, 8] in which graft survival and rejection frequency did not improve with CyA double therapy, and in which steroids, rather than AZA, was given systematically as the second drug. Even if the second drug were different, the fact that our patients were selected on the basis of a previous rejection episode might explain the difference, as they represent a population at high immunological risk, one that needs an extra immunosuppressive treatment. Including in the comparison patients who have never rejected and who do not need a second drug would only lessen the effect of AZA or steroids on rejection.

Polyclonal globulins or an anti-IL2 receptor monoclonal antibody were used in the period immediately after transplantation, and it could be argued that this may account for the differences in rejection recurrence. However, we have recently shown that the incidence of rejection is similar with these two therapies [1].

The addition of AZA did not increase the infectious risks in comparison to CyA monotherapy. Three deaths occurred in the patients receiving AZA, but they were not related to immunodepression. AZA itself had no serious side effects that limited its administration, and a tendency to macrocytosis was the only significant hematological abnormality noticed; however, long-term toxicity cannot yet be excluded.

Those who advocate the use of multiple therapies claim that combining several immunosuppressive drugs allows one to administer lower dosages of each and thus prevent their side effects. This is an important point in the case of CyA and its nephrotoxicity. Patients in both groups here received similar maintenance dosages of CyA, resulting in similar

CyA blood levels. Mean serum creatinine did not differ in recipients under monotherapy or double therapy; rather, as expected, patients in both groups who experienced more than one rejection after randomization had a lower renal function (but not significantly so) than those who had not rejected. Whether or not the decrease in CyA dosages, made possible by the introduction of AZA, will lead to reduced risks of CyA nephrotoxicity and to improved graft function, with the same protective effect against rejection, remains to be seen. The randomized studies by Griffin et al., that were referred to earlier [3], in which CyA monotherapy was compared with CyA-steroids double therapy, drew conclusions similar to our own. That is, the drug combination did not result in the CyA blood levels remaining lower, nor were there any significant differences in mean serum creatinine and CyA dosages between patients with and without steroids.

The choice of the second immunosuppressive drug can be debated. AZA has, in our experience, always had fewer side effects than steroids. In contrast, Salaman et al. [3, 8] have pointed out that corticotherapy accounted for dramatic specific morbidity, such as bowel perforations, and for minor ones, such as changes in facial appearance, that are dreaded by transplanted patients. A recent study [6] has reported the results of replacement of steroids by AZA in 25 primary cadaveric renal transplant recipients under CyA, and it has shown that after steroid withdrawal, body weight, mean blood pressure, and serum cholesterol concentrations were significantly lower. On the other hand, just one AZA-induced leukopenia necessitated its discontinuation. In our study, no more than one significant macrocytosis occurred, confirming our preference for AZA over steroids.

From the randomized trials referred to earlier, CyA-steroids double therapy does not seem to be superior to CyA monotherapy. Several groups [2, 10] are now using a triple therapy protocol, consisting of low dosages of CyA, steroids, and AZA. However, their studies have either not been randomized or, if controlled, as in a recent publication by Ponticelli et al. [9], in which the comparison was done with a CyA-AZA combination, they revealed that the double therapy yielded better results with regard to rejection frequency. It would thus appear that no matter what drug is combined with CyA, the risk of infection increases. Because of this and because of the potential risk of cancer that is always difficult to estimate at the moment, we think it wiser to add a second immunosuppressive drug only when indicated by the clinical events during and after transplantation. Indeed, the occurrence of rejection is the foremost reason for increasing immunosuppression. In that respect, although the number of patients included in our series is limited, the results, especially with regard to rejection recurrence, are clear-cut and significant. Although the severity and possible consequences of a first rejection episode are unpredictable, the systematic administration of AZA together with CyA seems to us to be a good alternative and has become our current strategy.

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