Sequential Therapy in Pediatric Cadaveric Renal Transplantation: A Critical Analysis

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ABSTRACT

Cadaveric renal transplant survival rates in children are still somewhat inferior to those reported in adults. Sequential therapy, an immunosuppressive program in which a course of antilymphocyte preparation is used immediately postoperatively and followed in sequence by oral maintenance immunosuppression, has a number of features that might be expected to improve pediatric transplant outcome by addressing some of the metabolic and immunologic difficulties encountered in children. This article examines the rationale for sequential therapy in pediatric renal transplantation. It also examines the use of sequential therapy in children. Sequential therapy appears to significantly improve cadaver renal allograft outcome in children without compromising patient survival. There was a slight improvement in 1- and 2-yr graft outcomes when OKT3 was used prophylactically when compared with antilymophocyte globulin, but this difference did not reach statistical significance. Almost 60% of patients receiving OKT3 for sequential therapy remained free of rejection. Major drawbacks of sequential therapy include the adverse side effects of the antilymphocyte preparation, infection, and the possibility of lymphoproliferative syndrome. The implications of these problems as well as potential strategies for ameliorating them are discussed.

Key Words: Sequential therapy, pediatric renal transplantation

Goals and Disadvantages of Sequential Therapy

Sequential therapy has a number of aims. It is used with the expectation that it will provide a rejection-free period in the days immediately after transplant surgery when the patient is least clinically stable. Graft dysfunction is quite common during this time, and it is sometimes difficult to recognize and treat significant rejection activity because the major manifestation of rejection, diminishing kidney function, cannot be discerned. By reliably blocking rejection or delaying its onset until it can be recognized, graft survival may be improved. In addition, the use of powerful induction immunosuppression might be expected to decrease the overall incidence of rejection. Finally, the use of a non-nephrotoxic induction agent...
allows the transplant team to avoid the use of high-dose CsA in the early posttransplant period. CsA exerts much of its acute nephrotoxicity by decreasing afferent arteriolar blood flow (3). It is likely that this effect can prolong the period of early renal dysfunction and ultimately impair allograft survival in a variable proportion of patients (3).

The use of sequential therapy also has a number of potential drawbacks. Antilymphocyte agents appear to increase the incidence of posttransplant infection, particularly cytomegalovirus (CMV) infection (4). If OKT3 is used as an induction agent, it is possible that anti-OKT3 antibodies may be generated (5); such antibodies could preclude its reuse (6). The use of an antilymphocyte preparation is also associated with a number of side effects that could complicate the posttransplant course (Table 1). One of the most ominous of these is the lymphoproliferative syndrome (7).

**RESULTS WITH OKT3 INDUCTION IN ADULTS**

As might be expected, the experience with induction therapy in adults is considerably larger than that in children. Recent studies in adults have compared the prophylactic use of OKT3 with regimens employing CsA from the outset, and it is possible to draw some conclusions from these studies. OKT3 induction appears to be associated with improved renal allograft outcome. In a multicenter controlled trial, 224 patients were randomized to receive either triple therapy (CsA, azathioprine, and prednisone) from the outset or OKT3 induction followed by triple therapy as maintenance immunosuppression. Prophylactic treatment with OKT3 resulted in a significantly smaller percentage of patients experiencing rejection and a significantly longer time from transplantation to an initial rejection episode (8). Patients who received OKT3 prophylaxis had an 8 to 9% improvement in graft survival rates at 6 months, 1 yr, and 2 yr ($P = 0.077$) (8). Similarly, Goldman et al. found that, when compared with CsA used from the outset, induction with OKT3 was associated with a significant increase in the number of patients who never experienced a rejection episode, a significant reduction in the total number of rejection episodes, and a significantly lower number of corticosteroid-resistant rejection episodes (9). In this study, as with the previous one, there was an improved graft survival, which was maintained at 18 months posttransplant (92% in the OKT3 group versus 79% in the CsA group) (9). On the negative side, Goldman et al. found that the use of OKT3 was associated with an increased incidence of benign infections during the first 3 posttransplant months (9). Nevertheless, actuarial patient survival rates were identical in both groups. It seems reasonable to conclude from these studies that in adults, sequential therapy with OKT3 confers some significant benefits.

One potential criticism of sequential therapy has been that it may result in increased costs. However, it is important to recognize that the most expensive transplant, in both financial and human terms, is the failed transplant. Manninen et al. (10) from the Battelle Research Institute showed that graft failure during the initial hospitalization resulted in considerably higher hospital costs than those incurred with a successful transplant. Similarly, graft failure after initial hospital discharge was associated with much higher follow-up hospital costs. In addition to these higher direct costs, graft failure increased a number of so-called "indirect costs" such as increased work disability, greater functional impairment, poorer health status, and a lower subjective quality of life. It should be acknowledged that some of these findings may differ in the pediatric situation. Nevertheless, the gains in graft improvement that may be realized from the use of sequential therapy are likely to result in cost benefits when viewed in this context. Even if we restrict ourselves to a strictly financial analysis, a careful examination of immunosuppressive medication costs over 5 yr reveals that there is little difference between dual drug therapy (CsA plus prednisone), triple therapy, or sequential therapy (R. Evans, personal communication) (Table 2).

**WHY MIGHT SEQUENTIAL THERAPY BE PARTICULARLY APPROPRIATE FOR CHILDREN?**

It has become increasingly evident that renal transplantation in children and adolescents differs sub-
stantively from transplantation in adults. In the CsA era, reported results of pediatric cadaveric renal transplantation have been variable and often inferior to results in adults. A report from the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) describes a 1-yr cadaver actuarial graft survival rate of only 72% in 385 children <18 yr old (11).

There are a number of biologic differences between children and adults that can affect the outcome of pediatric cadaver renal transplantation (12). Some of these differences can be ameliorated by sequential therapy (13) (Table 3).

Children metabolize CsA differently than adults, with more rapid equilibration and elimination kinetics (14). The half-life of CsA is age dependent (15), so young children often metabolize the drug much more rapidly than do older children. In addition, the pharmacokinetics of CsA are highly variable in children (16). CsA absorption may also differ between children and adults. CsA is dependent on the length of the small intestine; in young and/or growth-retarded children, CsA absorption may be unpredictably impaired because of variability in small bowel length (17). For all of these reasons, children often require higher CsA doses on a weight (i.e., milligram per kilogram) basis (12) and/or more frequent dosing intervals to achieve appropriate therapeutic blood levels. The provision of a rejection-free period, which sequential therapy affords, allows appropriate time to optimize maintenance immunosuppression and thus forestall rejection.

Small children present significant technical challenges in pediatric renal transplantation (18); because of their small size and technical complexity, reanastomosis times may be prolonged. Long reanastomosis time and resultant early allograft dysfunction with acute tubular necrosis may reduce allograft survival (19). The use of sequential therapy allows for a period of functional recovery before CsA is introduced.

Finally, there is increasing evidence that young children have heightened immunologic reactivity when compared with older children and adults. The immunologic defects often observed in adult dialysis patients appear to be absent in children (20). Young children on dialysis under 6 yr of age have higher indices of nonspecific cellular immune responsiveness than do older children or young adults. These indices include spontaneous blastogenesis; numbers of CD4-positive lymphocytes, B lymphocytes, and immature activated T lymphocytes; and an increased CD4/CD8 T lymphocyte ratio (21). Pediatric kidney and liver transplant recipients also show similar patterns. Young transplant recipients (<2 yr of age) have higher numbers of CD2+, CD3+, CD4+, and CD8+ lymphocytes than do older children; there is a downward trend in each of these lymphocyte subset populations until the age of 5 yr, when values are similar to those that have been reported in adults (22). All of the above tests, when increased, indicate an increased propensity for rejection (23,24). It is very possible that this increase in immunologic responsiveness and propensity for rejection is common to all young children, because increased numbers of CD4-positive T lymphocytes (25) and an increased CD4/CD8 ratio (25,26) are present in normal young children and vary inversely with age. This immunologic "hyperreactivity" makes sequential therapy appropriate for two reasons. It supplies augmented immunosuppression and tips the immunologic balance away from graft rejection. Simultaneously, it permits the necessary time to establish optimal maintenance immunosuppression.

<table>
<thead>
<tr>
<th>Table 2. Immunosuppressive drug costs over a 5-yr time period in adult renal transplantation</th>
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<tr>
<td><strong>Immunosuppressive Protocol</strong></td>
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<tr>
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<tr>
<td>Double Drug</td>
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<td>Triple Drug</td>
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<td>Quadruple Drug (i.e., Sequential Therapy)</td>
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<tr>
<th>Table 3. Sequential therapy: why might it be appropriate in children</th>
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<td><strong>Problem</strong></td>
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<tr>
<td>CsA metabolism and absorption are unpredictable in young children</td>
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<tr>
<td>Long reanastomosis times and early graft dysfunction with CsA leads to reduced graft outcome</td>
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<tr>
<td>Young children may have increased immunological responsiveness</td>
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<tr>
<td>2. Allow time to achieve optimal maintenance immunosuppression</td>
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THE USE OF SEQUENTIAL THERAPY IN PEDIATRIC RENAL TRANSPLANTATION

According to the recent NAPRTCS report, 45% of children receiving renal transplants in the United States from 1987 to 1989 received some form of induction therapy (11). Polyclonal preparations, i.e., ATG or MALG, were used six to seven times more frequently than OKT3. The median time course for induction therapy with a polyclonal preparation was 9 days, whereas for OKT3, it was 10 days. The median dosage for the polyclonal preparations was approximately 15 mg/kg/day; for OKT3, the median dose was 0.1 mg/kg/day.

In the NAPRTCS report, the use of induction therapy was associated with a significantly decreased rejection rate in the first 100 days after transplantation. However, as a general rule, induction therapy appeared to defer rather than decrease rejection episodes. By 1 yr posttransplant, there were equivalent rejection rates when comparing those who received induction therapy with those who did not (11). Induction therapy did not appear to significantly affect the probability of completely reversing later rejection episodes. Moreover, the NAPRTCS report published in 1990 found no improvements in the cumulative living related or cadaver donor graft survival rates associated with sequential therapy (11). However, analysis of more recent data from NAPRTCS employing a larger database has revealed a statistically significant improvement in the cumulative graft survival at 1 and 2 yr (27).

Single-center studies also suggest that some form of induction may improve graft survival in children. At the University of Minnesota, a fixed course of MALG has been shown to improve pediatric allograft outcome (28,29). The use of sequential therapy significantly improved patient and allograft outcome in 131 pediatric renal transplants (29). The 1- and 5-yr cumulative allograft survival rates in the 49 cadaver transplants in this study were 92 and 74%, respectively. The use of sequential therapy was particularly beneficial for recipients under the age of 6 yr, boosting the cadaver graft survival at 1 yr from 48 to 78% (28).

In July 1985, the pediatric immunosuppressive protocol at UCLA was redesigned to address the issues discussed above. A sequential therapy program was instituted with either ATGAM (Upjohn Pharmaceuticals, Kalamazoo, MI) or later OKT3 for the induction phase. AGTAM was used at a dose of 15 mg/kg/day, given through a central line. When ATGAM became unavailable, OKT3 was substituted. Initially, OKT3 was used at a dose of 2.5 mg/day in patients weighing less than 30 kg (N = 8) and 5 mg/day in patients weighing 30 kg or more (N = 14). In an attempt to reduce untoward “first dose” side effects, the first two doses of OKT3 were later reduced to 1 mg (N = 18). Prophylactic ATG or OKT3 was administered for a maximum of 14 days, or until the serum creatinine level fell to the range of 2 mg/dL. In addition to the antilymphocyte preparation, we administered prednisone (0.5 mg/kg with a minimum dose of 20 mg) and azathioprine (2 mg/kg); when OKT3 was given, low-dose CsA (5 to 6 mg/kg/day) was added to the regimen to decrease the development of anti-OKT3 antibodies. When the serum creatinine level fell to 2 mg/dL, CsA was initiated at full dose, i.e., 12 mg/kg or 500 mg/m² body surface area in children 6 yr of age and younger. Prednisone and azathioprine were continued. Care was taken to achieve therapeutic CsA trough blood levels (Table 4) before OKT3/ATG was discontinued. Often, OKT3/ATG was continued for 2 to 3 days while appropriate CsA therapeutic levels were established. Maintenance immunosuppression was carried out by adjusting medication doses to achieve the “therapeutic targets” shown in Table 4. Twelve-hour whole blood CsA trough levels were monitored at every clinic visit; in general, CsA whole blood levels were usually maintained in the upper bounds of the ranges shown in Table 4 (12,30).

In an earlier analysis of our results at UCLA, the adoption of sequential therapy was found to have made the largest and most significant contribution to improvement in primary cadaveric graft outcome in the Cox Proportional Hazards Model (13). The only other variable that was shown to have a significant effect was donor age; the use of donors over the age of 6 yr was associated with a significant improve-

<table>
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<th>TABLE 4. UCLA pediatric renal transplantation “therapeutic targets”—1991</th>
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<td><strong>Wk after Transplantation</strong></td>
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<tr>
<td>CsA whole blood trough level (ng/mL)</td>
</tr>
<tr>
<td>Polyclonal TDX</td>
</tr>
<tr>
<td>HPLC</td>
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<tr>
<td>Prednisone Dose (mg/kg/day)</td>
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<td>Azathioprine Dose (mg/kg/day)</td>
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ment in graft outcome (13). Table 5 shows the latest actuarial allograft survival rates for 76 primary and 25 retransplant recipients of cadaver grafts who have received sequential therapy at UCLA. For recipients of primary transplants, the 1- and 5-yr graft survival rates are 93 and 80%; for recipients of retransplants, the 1- and 3-yr graft survival rates are 84 and 64%. For both primary and retransplant graft recipients, these results at 1 yr are 20% higher than those reported by NAPRTCS (11). Within our primary cadaveric donor recipient group, the 1- and 2-yr cumulative allograft survivals with OKT3 are 95 and 91%; with ATGAM, the graft survivals at these same time periods are 91 and 87% (P = not significant).

Patient survival rates are identical between those treated with OKT3 and ATG (96% at 2 yr). Sequential therapy appears to be particularly beneficial in our young allograft recipients, in whom the problems identified above are the most vexing. The implementation of sequential therapy in children less than 6 yr of age has resulted in a dramatic improvement in graft outcome at UCLA—from a 1-yr cadaver graft survival of 33% with other forms of immunosuppression to 94% with the regimen outlined above (18,31). This has been achieved without any reduction in patient survival rates (31).

An in-depth analysis of 40 pediatric cadaver donor recipients receiving OKT3 induction therapy at UCLA revealed that only 5 (13%) experienced a rejection episode in the first posttransplant month. Fifty-nine percent of the patients remained rejection free, whereas 16 (41%) had 21 rejection episodes.

NEGATIVE ASPECTS OF SEQUENTIAL THERAPY IN PEDIATRIC RENAL TRANSPLANTATION

The use of agents, such as OKT3, that result in increased nonspecific immunosuppression has a number of important implications in pediatric renal transplantation. It would be imprudent to pursue a sequential immunosuppression strategy that improves allograft outcome if there emerged, as an additional result, an unacceptably high rate of infectious, immunologic, or malignant complications.

Table 6 classifies the adverse reactions seen in 40 children receiving OKT3 in the cadaveric sequential therapy protocol at UCLA. The severe early complications included cerebral edema, pulmonary edema, seizure, and arrhythmias. The episode of fatal cerebral edema unaccompanied by any central nervous system structural abnormality prompted us to reduce the first two doses of OKT3 in the induction period. Even with this measure, pulmonary edema was observed in one patient when her standard dose of 5 mg of OKT3 was given on the third day. The administration of high-dose corticosteroids 1 to 3 h before OKT3 administration is of some help in ameliorating some of the early adverse side effects (32). Other measures such as the use of indomethacin (33) or agents that block OKT3-mediated tumor necrosis factor alpha release (34) may be helpful in aborting these side effects.

As noted in Table 6, 15% of the pediatric transplant recipients at UCLA who received sequential therapy with OKT3 developed an infectious complication. Although a number of agents can be responsible for such infections after sequential therapy, CMV infection has emerged at UCLA as a particularly vexing problem. This is not surprising given the dependence on cadaveric donor transplantation at our center and the preference for cadaveric donors over 6 yr of age, because of the associated improved graft survival (13). CMV-seronegative patients receiving a kidney from a CMV-seropositive donor are at a significantly higher risk of serious disease compared with seropositive recipients. The acquisition of anti-CMV antibody increases with age; Broyer et al. found that only 28% of children under the age of 10 yr were seropositive, whereas almost 60% of those ages 16 to 20 yr had acquired antibody (35). Certain agents have been shown to be effective in preventing primary symptomatic CMV infections. Snydman et al. showed that CMV hyperimmune globulin given prophylactically decreased the severity of CMV disease in seronegative patients receiving ALG or OKT3 for rejection (36). Prophylactic oral acyclovir has also been used successfully in adult renal transplant recipients on sequential therapy (37). Yet another strategy is to use high doses of standard i.v. immune globulin (IVIG). Certain lots of standard IVIG have anti-CMV titers that are as much as half those of the hyperimmune preparations, and these may be effec-

<table>
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<th>Transplant No.</th>
<th>% Actuarial Graft Survival (N at risk) at Yr:</th>
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<tr>
<td>First (N = 76)</td>
<td>93 (56) 89 (39) 85 (26) 85 (15) 80 (4)</td>
</tr>
<tr>
<td>Retransplant (N = 25)</td>
<td>84 (20) 75 (14) 64 (7)</td>
</tr>
</tbody>
</table>

TABLE 5. Cadaver renal transplant actuarial survival in pediatric recipients of sequential therapy at UCLA—1991
TABLE 6. Adverse reactions in 40 children receiving OKT3 in the cadaveric sequential therapy protocol at UCLA*

<table>
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<tr>
<th>Early—Mild</th>
<th>Early—Severe</th>
<th>Infections</th>
</tr>
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<tbody>
<tr>
<td>Fever (33)</td>
<td>Cerebral Edema and Death (1)</td>
<td>Urinary Tract infection</td>
</tr>
<tr>
<td>Diarrhea (12)</td>
<td>Pulmonary Edema (3)</td>
<td>Candida (2)</td>
</tr>
<tr>
<td>Vomiting (8)</td>
<td>Seizure (2)</td>
<td>Bacterial (2)</td>
</tr>
<tr>
<td>Headache (9)</td>
<td></td>
<td>CMV Pneumonia (1)</td>
</tr>
<tr>
<td>Conjunctivitis (9)</td>
<td>Arrhythmia (1)</td>
<td>CMV Viremia and Disease (1)</td>
</tr>
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*Numbers in parentheses are number affected.

A particular concern with sequential therapy with OKT3 has been the possibility that such therapy may cause the generation of anti-OKT3 antibodies (38,39). Such antibodies can reduce or abrogate the ability of OKT3 to reverse steroid-resistant rejection episodes (38). The argument has been made that the use of OKT3 as prophylaxis dramatically reduces the incidence of steroid-resistant rejection episodes (39). In the 40 patients studied who received OKT3 sequential therapy at UCLA, 56% generated no anti-OKT3 antibody by ELISA testing, 25% had low-titer (i.e., 1:100) antibody, and 18% developed high-titer (i.e., ≥1:1,000) antibody. The combined figure of 43% for all antibody generation is less than the 75% quoted by Norman and Leone (38) and may represent the effect of the concurrent azathioprine and low-dose CsA we use during induction (39). Alternatively, differences could be due to the varying sensitivities of the different assay systems at different laboratories. Nevertheless, even a 43% incidence of anti-OKT3 antibody is of concern. Even low-titer antibodies make OKT3 reuse more difficult (often higher dosing schedules are required), and high-titer antibodies preclude the repeat use of OKT3 (and perhaps other mouse monoclonal antibodies).

The posttransplant lymphoproliferative syndrome (LPS) is a most ominous occurrence. The LPS in pediatric renal transplantation is reviewed elsewhere in this issue. Its occurrence has been linked to high doses of OKT3, at least in adult cardiac transplant recipients (7). We examined the total lifetime doses of OKT3 received by our pediatric sequential therapy recipients and found that only 2 (5%) of the 40 patients studied received more than the 75-mg total dose that Swinnen et al. identified as a contributing factor to LPS formation (7). Moreover, the total lifetime dose of OKT3 in our patients was 0.68 mg/kg, dramatically less than the 1.5 mg/kg calculated to have been received by the cardiac transplant recipients of Swinnen et al. (7). Even though none of our sequential therapy patients at UCLA have developed LPS, others have reported its presence in children (39). It is likely that the occurrence of posttransplant malignancies in general, and LPS specifically, is most closely related to the intensity of the immunosuppression used (40). Thus, even though one of the principles of sequential therapy is augmented immunosuppression, prudence and restraint are often indicated when treating recalcitrant rejection episodes.

CONCLUSION

Sequential therapy appears to be well suited to meet the demands of the pediatric cadaver renal transplant recipient. Its use appears to result in improved graft survival without any negative effect on patient survival. It is not a panacea, however. Appropriate precautions to minimize associated adverse side effects are clearly warranted. Perhaps even more importantly, diligent monitoring of the patient and...
his/her maintenance immunosuppression is still critical for long-term optimal graft outcome. Sequential therapy is not a "silver bullet," but only one way among many to give children a chance to have early optimal graft outcome.

REFERENCES


