Safety and Efficacy of a Calcineurin Inhibitor Avoidance Regimen in Pediatric Renal Transplantation

儿童肾移植中无 CNI 方案的安全性和有效性

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- Received for publication January 16, 2006.
- Accepted for publication March 25, 2006.

Next Section

Thirty-four children were entered into a pilot trial of calcineurin inhibitor avoidance after living-donor kidney transplantation, the CN-01 study. Patients were treated with anti-CD25 mAb, prednisone, mycophenolate mofetil, and sirolimus. Twenty patients were maintained on the protocol for up to 3 yr of

follow-up. One enrolled patient did not receive the transplant because of a donor problem, eight terminated because of one or more rejection episodes, four terminated because of adverse events, and one was lost to follow-up. Two grafts were lost, one as a result of chronic rejection and the other as a result of posttransplantation lymphoproliferative disorder. There were no deaths. The 6- and 12-mo acute rejection rates were 21.8 and 31.5%, respectively. GFR were stable throughout the course of the study, with a slight downward trend by 6 mo after transplantation followed by a slight upward trend to a mean of 70 ml/min thereafter. Early surveillance graft biopsies frequently showed focal interstitial mononuclear cellular infiltrates without overt vasculitis or tubulitis, but these infiltrates disappeared without treatment. Anti-HLA class I and II antibodies were detected in three patients before transplantation, and all three had acute rejections, including the two patients who lost their grafts. *De novo* anti-HLA Ab production occurred in only one patient after transplantation. There were two episodes of Epstein Barr virus–related posttransplantation lymphoproliferative disorder, one of which developed after the patient had been terminated from the study. It is concluded that calcineurin inhibitor–free immunosuppression can be safe and effective in pediatric living-donor renal transplantation. However, further modifications that are designed to lessen early rejection rates and decrease complications should be tested before this approach is used routinely.

Renal transplantation has long been considered the preferred treatment of ESRD in children (1–3). 长期以来，肾移植被认为是儿童终末期肾病的首选治疗方案。For years, however, children had poor outcomes and were considered to be high risk as compared with adults (4).但多年来儿童肾移植的治疗效果都比不上成人。 Improvements in donor selection, surgical techniques, and knowledge of immunosuppressive drug doses and metabolism in children have led to substantial improvements in pediatric kidney graft and patient survival (5,6). 随着供体选择水平和手术技术的逐步提升，以及免疫抑制剂相关理论知识的不断丰富，儿童肾移植的移植物存活率和受体存活率得到了显著提升。The improvement in outcomes n children have exceeded those of adults, and children who are younger than 10 yr now have the best outcomes of all age groups of kidney transplant recipients (6).治疗效果的进展速度已经超过了成人，如今，小于 10 岁的受体治疗效果是所有年龄别中最好的。

Much of the improvement in organ transplantation outcomes has been due to the prevention of early acute rejection episodes and prompt identification and treatment when they occur (5,7–9).这样的进展大部分要归功于对早期急性排斥反应的预防、诊断以及治疗上的进步。Whereas some single-center reports have described rates of acute rejection episodes as low as 13 to 26% at 1 yr in selected groups of pediatric recipients (10–13), large multicenter studies have reported rates as high as 27 to 59% (14–19). Registry studies have reported that overall 6-mo acute rejections rates of in deceased-donor and 32% in living-donor kidney transplantsin 1992 fell to 21 and 20%, respectively, by 2003 (20). The North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) reported a 57% first-year acute rejection rate in pediatric renal transplant recipients in 1987 that decreased to 32% by 2001 (5).单中心试验报道的 1 年内急性排斥反应发生率在 13%到 26%之间，而大型的多中心试验报道的在 27%到 59%之间。注册研究已经报道，1992 年 6 个月内急性排斥反应发生率在接受死者捐献的患者中是 45%，到 2003 年下降到 21%，而 1992 年接受生者捐献的患者中是 32%，到 2003 年下降到 20% 。

One of the major reasons for decreased acute rejection rates and improved short-term outcomes is the improvement in immunosuppressive medications (21,22). 急性排斥发生率下降和短期结局提高的主要原因之一是免疫抑制药物的进步。Among these, the calcineurin inhibitors (CNI) are the most potent and likely the most important in improving outcomes (23). 在这些药物当中 CNI 可能是最强大和最重要的进步。However, these medications have multiple adverse effects, among which the most significant is nephrotoxicity (24-27). 但是 CNI 类药物具有多重副作用，其中肾毒性最为引人注目。Chronic CNI-associated nephrotoxicity is thought to be one of the antigen-independent factors related to the progression of chronic allograft nephropathy (CAN). 慢性 CNI 相关性肾毒性被认为是 CAN 发展的非免疫学危险因素。Protocol biopsies of pediatric kidney transplant recipients have demonstrated interstitial fibrosis and tubular atrophy characteristics of CNI nephrotoxicity (28,29). 儿童肾移植的计划性活检显示间质纤维化和小管萎缩是 CNI 肾毒性的特征。Importantly, as many as 15% of extrarenal transplant recipients develop chronic renal insufficiency, and CNI toxicity is thought to play a major role in the development of that disorder (30). In addition, CNI have been associated with hypertension, increased rates of steroid-associated diabetes, and neurologic complications (31).15%的其他器官移植患者罹患慢性肾功能不全，CNI 的毒性在此起主要作用。此外，CNI 也与高血压、类固醇性糖尿病、神经系统并发症相关。

There is very little experience with the use of the target of rapamycin (TOR) inhibitors sirolimus and everolimus in pediatric organ transplantation (32-36). 在儿童器官移植中有关雷帕霉素抑制剂西罗莫斯和依维莫司应用的经验很少。This class of immunosuppressants has a novel mechanism of action that is different from all other antirejection medications that are used for transplantation (37,38). 这一类免疫抑制剂具有新型的作用机制。The major complications of the TOR inhibitors nclude hyperlipidemia, thrombocytopenia, and poor wound healing (38). 它们的主要副作用是高脂血症、血小板减少和伤口难愈。Sirolimus has been used in adult kidney transplant recipients in CNI avoidance or withdrawal studies (39).西罗莫斯作为无 CNI 方案已经应用于成人的肾移植中。 These studies generally have demonstrated better long-term GFR in recipients in whom there is CNI avoidance or withdrawal as compared with those who receive chronic CNI-based immunosuppression. 那些研究多数证明了无 CNI 方案长期对保持 GFR 有好处。但短期的急性排斥发生率比较高。However, acute rejection rates may be somewhat higher. Because of these early results in adult renal transplant trials, we undertook a pilot study of CNI avoidance in pediatric renal transplantation.因为成人身上的这些结论，我们在儿童肾移植群体中实施了类似的研究。

Previous SectionNext Section

长期以来，肾移植被认为是儿童终末期肾病的首选治疗方案。但多年来儿童肾移植的治疗效果都比不上成人。随着供体选择水平和手术技术的逐步提升，以及免疫抑制剂相关理论知识的不断丰富，儿童肾移植的移植物存活率和受体存活率得到了显著提升，治疗效果的进展速度已经超过了成人。如今，小于 10 岁的受体治疗效果是所有年龄组别中最好的。这样的进展大部分要归功于对早期急性排斥反应的预防、诊断以及治疗上的进步。单中心试验报道的 1 年内急性排斥反应发生率在 13%到 26%之间，而大型的多中心试验报道的在 27%到 59%之间。注册研究已经报道，1992 年 6 个月内急性排斥反应发生率在接受死者捐献的患者中是 45%，到 2003 年下降到 21%，而 1992 年接受生者捐献的患者中是 32%，到 2003 年下降到 20% 。急性排斥发生率下降和短期结局提高的主要原因之一是免疫抑制药物的进步。在这些药物当中

CNI 可能是最强大和最重要的进步。但是 CNI 类药物具有多重副作用，其中肾毒性最为引人注目。慢性 CNI 相关性肾毒性被认为是 CAN 发展的非免疫学危险因素。儿童肾移植的计划性活检显示间质纤维化和小管萎缩是 CNI 肾毒性的特征。15%的其他器官移植患者罹患慢性肾功能不全，CNI 的毒性在此起主要作用。此外，CNI 也与高血压、类固醇性糖尿病、神经系统并发症相关。在儿童器官移植中有关雷帕霉素抑制剂西罗莫斯和依维莫司应用的经验很少。这一类免疫抑制剂具有新型的作用机制。它们的主要副作用是高脂血症、血小板减少和伤口难愈。西罗莫斯作为无 CNI 方案已经应用于成人的肾移植中。那些研究多数证明了无 CNI 方案长期对保持 GFR 有好处。但短期的急性排斥发生率比较高。因为有在成人身上的这些结论，所以我们在儿童肾移植群体中实施了类似的研究。

Materials and Methods

Study Design and Patient Enrollment 纳入标准：活体捐献+<21 岁；设计：单组预试验；方案：达利珠单抗诱导+泼尼松+MMF+西罗莫斯；观察时限：移植后 1 年，随访三年；统计指标：不良反应事件、移植物活检、GFR；

The Cooperative Clinical Trials in Pediatric Transplantation (CCTPT) is a cooperative research program sponsored by the National Institute of Allergy and Infectious Diseases. Four participating centers of CCTPT entered patients into this study, identified as CN-01 study, which was designed as a single-arm pilot study in which all patients received daclizumab induction, prednisone, mycophenolate mofetil (MMF), and sirolimus. The primary objective of the study was to determine whether the rejection risk was low enough in the first year after transplantation to permit the use of chronic CNI-free immunosuppression in pediatric transplantation.在移植后一年的排斥反应发生率是否足够低以至于能够使用无 CNI 的方案。Adverse events, surveillance graft biopsies, and changes in measured GFR were monitored carefully. Living-donor kidney transplant recipients who were younger than 21 yr and receiving their first or second graft were eligible. The study protocol was approved by the institutional review board at each study site as well as by the National Institute of Allergy and Infectious Diseases. Informed consent was obtained from the patients who were 18 yr and older and from the parents of patients who were younger than 18 yr before their enrollment in the study. Patients who were older than 12 yr also provided assent.

Immunosuppression

All patients received a loading dose of sirolimus 1 d before transplantation. 移植前一天服用一次西罗莫斯。They received the anti-CD25 mAb daclizumab intraoperatively and every 2 wk up to 8 wk after transplantation. 在术中应用达利珠单抗，并且术后每两周再用一次，总共四次。Maintenance immunosuppression consisted of prednisone, which was begun at 2 mg/kg per d and tapered to 0.15 mg/kg per alternate day 强的松起始剂量 2mg 每公斤体重每天，逐渐减量至隔日 0.15mg 每公斤体重; MMF at a dose of 1200 mg/m^2 per d, divided twice daily;MMF1200mg 每平方米体表面积每天，分两次服用 and sirolimus, which was administered on a twice-daily schedule and at a dose that was designed to maintain whole-blood trough levels of 20 to 25 ng/ml for the first 2 mo 西罗莫斯前 2 月血药浓度 20-25ng/ml，3-6 月 20 ng/ml, 20 ng/ml for months 3 to 6, and 15 ng/ml thereafter. 之后就是 15 ng/ml 。Patients were followed for 3 yr.

Graft Evaluation

GFR were measured by elimination of radiolabeled technetium at 3, 6, 12, 24, and 36 mo after transplantation. GFR 是用放射性标记锝的排泄率来测定的，分别在移植后 3、6、12、24 月来测定

的。Surveillance kidney transplant biopsies 监视性肾移植活检 were obtained at the time of the transplant procedure (postperfusion) and at 3, 6, and 12 mo after transplantation. 是在移植后 3、6、12 月获得的。Two cores were obtained, one of which was used for routine processing 其中一个是用作常规检查 and review at the clinical center 和归档. The other core was split into two pieces, 第二个标本被分成两份 and both were snap-frozen in liquid nitrogen 都被液氮冷冻 and stored for later analysis. 并保存起来用作往后的分析 One piece was used for mRNA analysis, and the other was used for immunohistologic analysis.这两份一份被用作 mRNA 分析，另一份被用作免疫组化分析。

Alloantibody Detection

Blood samples for alloantibody detection 用作同种异体抗体检测的血液标本 were obtained 是在移植之前，移植后 3、6、12 月以及怀疑排斥发生时获得的。 before transplantation; at 3, 6, and 12 mo after transplantation; and at the time of suspected rejection. A flow cytometric Luminex XY platform 流式细胞仪被用作检测 class I 和 class II 抗体 was used to detect class I and class II alloantibody (OneLambda, Inc., Canoga Park, CA). Microbeads（免疫）微球是被纯化的 class I 和 class II HLA 抗原包被的，这些抗原代表所有的常见和许多罕见的抗原。 are coated with purified class I and class II HLA antigen that represents all common and many rare antigens. Test serum 在检测之前用作检测的血清一直被保存在−80°C。 was maintained at −80°C until just before testing. Serum samples were centrifuged at 8000 rpm for 5 min 血清标本在 8000 rpm 下离心 5min， to remove aggregates and tested undiluted. A total of 20 μl of test serum, as well as positive and negative control sera, was incubated with 5 μl of LABScreen class I and class II beads. One microliter of 100× conjugated anti-human IgG per test sample was diluted in 99 μl of wash buffer and incubated with the beads for an additional 30 min. All samples were analyzed within 1 h of completion. The LABScan analyzer with LABScreen analysis software was used for data acquisition. Serum reactivity was assessed by the PE fluorescence shift for each HLA-coated bead after correction for nonspecific binding to the negative control bead. Reactions are graded as positive, negative, or gray zone depending on the reactivity of the sample serum in comparison with the control serum.

Statistical Analyses

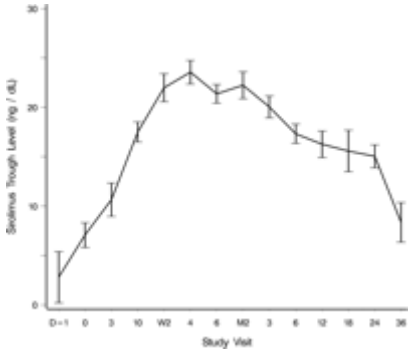
Statistical analysis was designed to determine whether the acute rejection rate was acceptable to permit subsequent studies. 按照设计，数据分析是用来计算急性排斥反应发生率是否足够低以至于能够进行一下研究的。We proposed that a rejection rate in the first 6 mo of 40% would be unacceptable and that a rate <20% would be desirable 我们设定急性排斥反应发生率在移植后 6 个月内超过 40%就不能进行下一步，而小于 20%是非常良好的, especially if the patients were free of CNI adverse effects.？？ 特别是当病人们没有发生 CNI 相关的 Therefore, if there were sufficient evidence to conclude that the acute rejection rate was <20 or >40%, then the study would have been terminated. The sequential testing procedure used was a truncated extension of the sequential probability ratio test. With the study sample size of 35, the design type I and type II error rates were 8 and 12%, respectively. The time course of repeated lipid and GFR measures was analyzed using generalized linear models with parameters estimated using generalized estimating equations.

Previous SectionNext Section

Results

Enrollment and Primary Outcome

Thirty-four patients were enrolled between February 2001 and August 2003. One enrolled patient did not receive the transplant because of a problem uncovered in the donor. 33 个入组 Six patients had at least 3 yr of follow-up, and 91% had at least 1 yr of follow-up（30 个人随访满 1 年）; the mean follow-up was 2.1 yr. Of those 33, nine were younger than 6 yr, four were 6 to 12, and 20 were older than 12. Twenty-one were boys, and there were no black patients. Ten patients received preemptive transplants 优先移植, two had previously received a kidney transplant, and three donors were unrelated. There were no episodes of delayed graft function. Patients received sirolimus on a twice-daily schedule (35), and target levels were achieved by the second week and maintained for the rest of the study (Figure 1).



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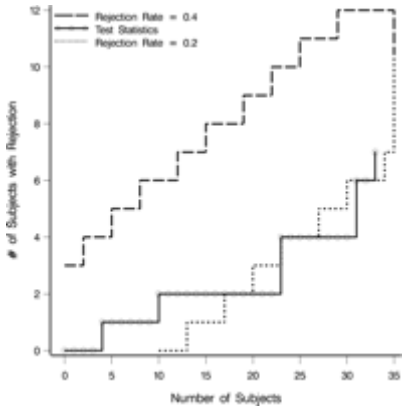
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Figure 1.

Mean and SE sirolimus trough levels from the time of transplantation until 36 mo after transplantation in children in CN-01 study.

Eleven patients had 14 acute rejection episodes, 11 个病人发生了 14 次急性排斥反应 seven within the first 6 mo and four after the first 6 mo.其中 7 人在六个月内，4 人在六个月后。 The plot of actual acute rejection rate in the first 6 mo *versus* sequential probability ratio test boundaries is shown in Figure 2. The test statistic did not approach the upper bound and remained close to the lower bound throughout the study. Two grafts were lost, one at 94 d as a result of recurrent rejection and posttransplantation lymphoproliferative disorder (PTLD) and the other at 732 d as a result of chronic rejection. 两个移植肾损失，1 个因为反复急性排斥和移植后淋巴增生性障碍在第 94 天时丧失，另 1 个因慢性排斥在第 732 天时丧失。There were no deaths.无死亡 Thirteen (39%) patients withdrew from the study treatment protocol (Table 1). 13 个病人退出此研究。Of these, eight were withdrawn because of one or more rejection episodes, four were withdrawn because of adverse events or complications, and one was lost to follow-up.其中 1 个是失访，8 个因为排斥反应，4 个因为并发症。 Of those with rejection, two lost their grafts and six still have functioning grafts after treatment of rejection. 8 个发生排斥反应而退出的患者中 2 个损失了移植肾，6 个经治疗保留了下来。In five of these cases, maintenance immunosuppression was changed by discontinuing MMF and replacement with tacrolimus. In one case, the patient was left on the study protocol of sirolimus, MMF, and steroids; that patient has not had any more episodes of acute rejection. Of the four patient who were withdrawn because of adverse events, two had neutropenia and one had diarrhea and vomiting; all three of these had resolution of symptoms when tacrolimus was substituted for MMF. One patient had a lymphocele and poor wound healing that

necessitated the temporary discontinuation of sirolimus; he is currently receiving sirolimus, tacrolimus, and prednisone.



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Figure 2.

Stopping guideline as determined by sequential probability ratio test. The upper dotted line indicates a rejection rate inconsistent with the lower 20% bound in the first 6 mo after transplantation, the lower dotted line indicates a rejection rate inconsistent with the 40% bound, and the solid line indicates the rate in CN-01 study.

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Table 1.

Reasons for withdrawal from study

Acute Rejections

As noted above, 11 patients experienced 14 episodes of biopsy-proven acute rejection. The incidence of acute rejection is shown in Figure 3. The 6-mo and 1-yr rates determined by Kaplan Meier estimates were 21.8 and 31.5%, respectively. Three of these episodes were identified in surveillance biopsies; 11 were classified as acute cellular, one as acute cellular/vascular, and one as acute and chronic rejection. In 12 cases of rejection, the serum creatinine was <2.0 mg/dl at the time of diagnosis. One of the two instances of rejection that were diagnosed with a creatinine >2.0 mg/dl was in the patient with chronic rejection. Both patients with previous transplants had pretransplantation alloantibodies, and both had acute rejection episodes. All rejection episodes were treated with Solu-Medrol pulses, and five also received Thymoglobulin for persistent (n = 4) or concomitant (n = 1) vascular rejection. All episodes responded to the treatment and resulted in stabilized or lowered creatinine concentrations except for the patient who had chronic rejection, who went on to lose her graft 2 mo later.

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