Toxic effects of immunosuppressive drugs: mechanisms and strategies for controlling them

LESLEY M. SHAW,1 BRUCE KAPLAN,2 and DIXON KAUFMAN2

Since cyclosporine (CsA) was introduced into clinical practice in late 1983 to prevent rejection in transplant patients, there has been an almost explosive growth in the number and types of transplants and the number of transplant centers, an increase in the life expectancy of the transplanted organ, and substantial decreases in rates of acute rejection and life-threatening infections. Despite these successes, major improvements in immunosuppressive therapy are needed, especially a reduction in toxic side effects and a rigorous definition of the relation between drug concentration and clinical effects. Such improvements may be achievable with the incorporation of new drugs such as tacrolimus and mycophenolate mofetil into immunosuppression protocols and the development of rigorously defined therapeutic drug-monitoring programs.

INDEXING TERMS: transplant patients • therapeutic drug monitoring • randomized concentration-controlled trial

The regulation of immunosuppressive therapy is the most critical aspect of aftercare in transplant patients. Too much immunosuppression increases the risk of side effects unique to each agent and of "immunotoxic" effects such as opportunistic infections and malignancy. Too little immunosuppression increases the risk of rejection and graft loss. Here we assess the progress made during the past 13 years, the so-called "cyclosporine (CsA) era," as the result of incorporating better immunosuppression regimens and CsA therapeutic drug monitoring, and we discuss the pursuit of improvements in transplant patient therapy today. The discovery and evaluation of new immunosuppressants, alone and in combination, are now underway at an explosive rate [1, 2]. Because of these advances it should be possible to take immunosuppressive therapy to the next level, providing the transplant patient an even lower risk of rejection and also further reducing risk for deleterious side effects.

Progress During the CsA Era

CsA was introduced into clinical practice as a primary immunosuppressant for rejection prophylaxis in transplant patients in late 1983. Thereafter CsA-based immunosuppression rapidly became the standard of practice worldwide. In the summer of 1984, Myers et al. [3] reported on the development of irreversible kidney damage in several heart transplant patients who received what is now referred to as "high-dose" CsA-based immunosuppression. This report led to studies of the mechanism of nephrotoxicity and the development of strategies for reducing the risk for its development in transplant patients while retaining effective immunosuppression. It is important to consider this side effect and efforts to curtail it because this led to major changes in CsA-based therapy and the search for less toxic immunosuppression.

Among the advances made during the past 13 years of CsA-based immunosuppression in transplant patients are [4]: (a) improved patient outcome including (i) decreased intensity and rate of rejection, (ii) decreased rate of life-threatening infections, (iii) better control of toxic effects, and (iv) decreased time spent in the hospital; (b) a substantial increase in the number and type of transplants; (c) a substantial increase in the number of transplant centers; and (d) the incorporation of a therapeutic drug-monitoring strategy for optimizing CsA dosing [5-12].

Despite progress in providing optimal immunosuppression to transplant patients, important improvements or therapeutic goals for transplant patients are needed. These suggested improvements reflect the collective thinking of the transplant community [13, 14] and are summarized in Table 1. These improvements in immunosuppressive therapy should result in substantially improved patient outcome, decreased costs, and fewer days in the hospital. Thus, in today's healthcare arena, we are at a pivotal time for meeting the challenge to provide ever more cost-effective care for transplant patients.
Table 1. Therapeutic goals* for transplant patients.
1. Reduction in the incidence of acute and chronic rejection
2. Reduction in the use of antirejection medications and associated costs
3. Reduction in the incidence and severity of debilitating side effects of immunosuppressive agents
4. Avoidance of overimmunosuppression
5. Development of rigorous pharmacokinetic–pharmacodynamic studies in clinical trials—basis for therapeutic drug monitoring
6. Development of patient-specific measurement of immunosuppression
7. Increased compliance
8. Reduction or elimination of chronic steroid therapy
9. Development of safe and effective therapy for autoimmune disorders

*All of these involve improved patient outcome, decreased costs, and decreased number of days in the hospital.

Side Effects of Immunosuppressive Drugs

All immunosuppressants have associated side effects. The most important or the most widely studied side effects of immunosuppressive drugs in current use are summarized in Table 2. As noted, the nephrotoxic effects of CsA played a major role in the development of subsequent immunosuppression protocols, and tacrolimus is also nephrotoxic [15]. Thus, a consideration of the mechanisms of CsA nephrotoxicity and successful strategies for minimizing this side effect should provide a basis for the more efficient development of tacrolimus-based immunosuppression and the successful incorporation of other new immunosuppressive drugs into clinical practice.

CsA and Tacrolimus Nephrotoxicity

RENAI FUNCTION AND MORPHOLOGY CHANGES

Acute changes. The hallmark acute renal hemodynamic changes caused by CsA include reduced renal blood flow, afferent arteriolar vasoconstriction, a decreased glomerular filtration rate (GFR), and increased renal vascular resistance [16]. The major acute renal tubular abnormalities attributable to CsA are decreased potassium secretion, decreased uric acid excretion, and increased magnesium excretion [17]. These acute changes increase serum concentrations of creatinine, urea nitrogen, potassium, and uric acid, and decrease serum concentration of magnesium [17]. If these nephrotoxic changes are not checked by appropriate CsA dosage reduction, characteristic morphological changes may occur, the most specific of which is hyalinization of afferent arterioles [18].

Chronic changes. Patients exposed to high blood concentrations of CsA for sustained periods of time may exhibit the following irreversible vascular changes: continued narrowing of afferent arterioles; damage to the endothelium and smooth muscle; arteriolar occlusion; vessel obliteration; glomerular dropout; tubular atrophy; and striped interstitial fibrosis [19]. These changes are largely preventable by adjusting CsA dosage early after transplant surgery to the currently accepted target blood concentration ranges [19, 20]. These changes were seen in patients exposed to the high-dose CsA regimens in use when CsA was first introduced into clinical practice but are less frequently seen in patients receiving the current low-dose CsA-based immunosuppression regimens with dosage guided by therapeutic drug monitoring [19]. Tubular changes in patients exposed to high CsA doses chronically are single cell necrosis, giant mitochondria, and vacuolization [19]. These changes can be reversed by reducing the CsA dose to achieve therapeutic blood concentrations [21].

Although far fewer studies of the detailed renal functional changes caused by tacrolimus have been done, Steinmuller [22] and Jusko et al. [23] concluded that these are essentially the same as the changes produced by CsA and that their occurrence should be minimized by a therapeutic drug-monitoring strategy.

MECHANISMS OF CSA NEPHROTOXICITY

The overall mechanism responsible for the development of chronic changes in kidney structure and, consequently, function is incompletely understood. CsA administration almost immediately reduces renal blood flow and GFR [16], as observed in animal models, in transplant patients, and in patients with autoimmune disorders [17]. In psoriasis patients, the biochemical changes described above correlated well with blood concentrations of CsA measured specifically [17]. The latter study is of special interest in that the observed effects can more directly be attributed to CsA because there were fewer confounding factors such as prior impaired kidney function and other medications that might alter kidney function; the investigators also used a parent-drug-specific method (HPLC) for drug measurement [17]. Hemodynamically, the hallmark event causing the reduced renal blood flow and GFR is believed to be the constriction of afferent arterioles to a greater extent than efferent arterioles [16]. Of the many reported biochemical changes associated with this acutely altered GFR, which ones are ultimately responsible for the hemodynamic changes is unclear. Possible mediators include increased thromboxanes and endothelin; enhanced sympathetic activity, platelet-activating factor, and angiotensin II; impaired nitric oxide production; and decreased vasodilating prostanooids [24]. It is not clear whether the acute hemodynamic effects of CsA cause chronic structural changes [25]. Chronic
renal histopathological changes include hyalinization of afferent arterioles, damaged endothelium and smooth muscle of afferent arterioles, tubular atrophy, glomerular sclerosis, and striped interstitial fibrosis. Burdmann et al. [25] found in recent animal model studies that these chronic changes may be dependent on angiotensin II but independent of the renal hemodynamic changes.

CSA Metabolites and Nephrotoxicity

Christians and Sewing [26] detected >30 metabolites of CsA. The structures of 15 of these have been characterized. Some metabolites appear in appreciable concentrations in tissues and blood in transplant patients, especially during episodes of liver dysfunction [27]. Christians et al. [27] reported an association of high concentrations of metabolites AM1c9 and AM19 with nephrotoxicity in the early postoperative period in some liver transplant patients but could not distinguish between cause and effect for the metabolites. Several consensus groups have concluded that the clinical importance of CsA metabolites is controversial and that in most clinical situations monitoring metabolite concentrations in patients' blood, in addition to that of the parent drug, is not warranted [7, 9, 10]. Nevertheless, the possible role of CsA metabolites remains under investigation [26]. Christians and Sewing [26] provide details of most studies conducted in this area to date.

Tacrolimus Metabolites

Tacrolimus is converted to at least 15 metabolites by several members of the cytochrome P-450 family [23, 28]. Less than 1% of the parent drug is excreted unchanged in urine [23, 28]. In single dose studies, tacrolimus metabolites do not accumulate appreciably; but in chronically dosed patients, five main metabolites, measured by HPLC–mass spectrometry, accumulated in blood as high as 145% and 152% of the tacrolimus concentration in liver and kidney transplant patients, respectively [29]. Jusko et al. [23] characterized the immunological activity of nine metabolites and measured cross-reactivity with the monoclonal antibody used in the commercially available tacrolimus immunoassays. However, nothing has been published regarding the possible toxicity of tacrolimus metabolites.

Strategies to Reduce the Risk for Developing Nephrotoxicity

Effective strategies for reducing the risk of acute and chronic nephrotoxicity are: (a) CsA dosage adjustment to maintain blood concentrations of the parent drug within a narrow target range. In three studies overt acute nephrotoxicity was reduced to an incidence of 4–8% by maintenance of parent-drug blood concentrations within a tight concentration range in renal transplant patients [8, 30, 31]. This contrasts sharply with the incidence of acute nephrotoxicity of 70% [32] and 370% [33] in renal transplant patients whose CsA dosing was based on serum creatinine changes alone. Although prolonged exposure to controlled-dose CsA causes some loss of renal function [34], renal function is stable and graft loss due to the chronic nephrotoxic sequelae, suggested by Myers et al. [3] under high dose CsA regimens, is avoided [35–37]. (b) Concomitant therapy with calcium channel-blocking antihypertensive agents is another strategy to decrease nephrotoxic risk. These agents attenuate some of the CsA-mediated renal hemodynamic changes but may not affect the more chronic histological changes [38]. (c) A risk factor for CsA nephrotoxicity is the underlying condition of the kidney itself [21]. Thus, the condition of the kidney before transplant surgery, the concomitant use of nephrotoxic agents, and the number and intensity of rejection episodes are examples of risk factors that predispose this organ to CsA nephrotoxicity. (d) Introducing new drugs, such as mycophenolate mofetil and rapamycin, with fewer overt side effects such as nephrotoxicity, can allow further reduction of CsA dosage but could also prompt a tendency to give more immunosuppressive drug than necessary. Thus, defining an upper window for reduced risk for immunotoxic effects is critical to providing optimal therapy and to rigorously defining relationships between drug concentration and risk for side effects.

Immunotoxicity

Adjusting immunosuppressive drug dosage to achieve narrow target concentration ranges can also reduce the risk for immunotoxicity. Besides drug-specific side effects, all immunosuppressive agents are immunotoxic, increasing risk for infections and cancers. The risk for infections has been successfully reduced by a combination of prophylactic anti-infective agents and tight control of CsA and tacrolimus dosing. All immunosuppressive agents put patients at increased risk for cancer.

PTLD is one of the most common cancers associated with immunosuppression [39, 40]. The incidence of PTLD is a function of the intensity of immunosuppression (Table 3). The mechanism responsible for the development of PTLD is postulated to be the loss of normal T cell surveillance secondary to immunosuppression. The risk for development of PTLD increases with increased intensity of immunosuppression and appears to be highest in transplant recipients with negative Epstein-Barr virus titers at the time of transplant surgery [41]. The pediatric patient population has a particularly high risk for this complication.

Strategies for Reducing PTLD Risk

Azathioprine and OKT3 subject transplant patients to the greatest risk for PTLD according to epidemiological studies [39]. Two important outcomes of the multicenter clinical trials for tacrolimus and mycophenolate mofetil were the reduction of OKT3-based full-course antirejection therapy by twofold and

<table>
<thead>
<tr>
<th>Table 3. Incidence of PTLD as a function of Immunosuppression Intensity [39].</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>immunosuppression</strong></td>
</tr>
<tr>
<td>Pre-1983, &quot;conventional&quot; immunosuppression</td>
</tr>
<tr>
<td>Early high-dose CsA</td>
</tr>
<tr>
<td>CsA, low-dose, guided by TDM</td>
</tr>
<tr>
<td>Triple therapy (CsA + steroid + azathioprine)</td>
</tr>
<tr>
<td>OKT3</td>
</tr>
<tr>
<td>*TDM, therapeutic drug monitoring; OKT3, orthoclone OKT3, muromonab CD3.</td>
</tr>
</tbody>
</table>


the elimination of azathioprine from triple therapy regimens for liver and renal transplant patients, respectively [15, 42]. The risk for PTLD could be lessened further by reducing or eliminating these two risk factors in the immunosuppression regimen and by further optimizing the new immunosuppression regimens guided by careful therapeutic drug monitoring strategies. Another important benefit of the reduction in acute rejection rate with new immunosuppression regimens is the decrease in number of hospital days and costs [42, 43].

New Immunosuppressive Drugs
The development of new immunosuppressive agents led to the recent introduction of tacrolimus and mycophenolate mofetil into clinical practice. Rapamycin is under clinical evaluation in renal transplant patients [44]. Leflunomide analogs, which inhibit the lymphocyte proliferative response primarily by interruption of de novo pyrimidine nucleotide biosynthesis [45] and humanized monoclonal antibodies such as CTLA4-Ig [46], which act by blocking the CD28-mediated costimulatory signal, are under intense investigation in animal transplant models. New agents and combinations of new and established immunosuppressive drugs will likely be used clinically in the near future. Thus, it might be possible to reduce further the risk for toxic effects of such drugs as CsA and tacrolimus if lower doses and concentrations will provide at least equivalent efficacy when combined with the newer agents, providing both a challenge and an opportunity to the laboratorian. In this scenario, it is important to develop well-founded therapeutic drug-monitoring strategies at the earliest possible time during drug development. Table 4 gives a summary of proposed therapeutic drug-monitoring goals for immunosuppressive drugs, based on the accumulated experience with monitoring CsA and tacrolimus. Continuing experience with CsA and tacrolimus clearly indicate the value of developing well-validated analytical methods for measuring active drug that meet stringent but realistic criteria [7, 9, 10, 47, 48].

Peck et al. [49] proposed that the randomized, concentration-controlled clinical trial (RCCT) can be an efficient way to acquire drug concentration vs. clinical effect information for specific cohorts of study patients. In the discipline of therapeutic drug monitoring, most of the guidelines for how to use drug concentration in the appropriate biological fluid have been developed through single-center retrospective review of concentration–effect data. The RCCT approach differs in that it is a prospective study design in which study patients are randomly assigned to one of several predetermined cohorts. The dosage is titrated for each patient cohort within a study to achieve a certain active drug steady-state concentration range for a sustained period. Hard outcome variables are measured throughout the study. For transplant patients, the outcome measures include acute rejection, toxic side effects, and infections. Drug concentrations in the appropriate biological fluid are measured at predetermined times during the study period. An example of this strategy is a double-blinded RCCT involving 120 renal transplant patients receiving mycophenolate mofetil together with CsA and prednisone. CsA dosage was regulated according to a trough concentration strategy, and prednisone was given according to an empirical dosing strategy. The basis for this study was the observation of a strong inverse correlation between the 12-h mycophenolic acid area under the concentration curve and risk for acute rejection [50–52]. The latter observation was originally made in a group of Japanese renal transplant patients and confirmed in a group of patients enrolled in the American multicenter trial [50]. In the RCCT study, the patients were assigned to one of three study groups, one receiving low doses of mycophenolate mofetil to achieve a low mycophenolic acid concentration range, the second a mid-target range, and the third a high target range. We hope that the results of these studies will provide a more rational basis on which to develop a therapeutic drug-monitoring strategy in individual patients. The clinical laboratorian can affect the development of such strategies by providing laboratory support and participating in the development of immunosuppression protocols.

Table 4. Therapeutic drug monitoring goals for immunosuppressive drugs.

| 1. Develop specific, well-validated methods for measurement of drug and active metabolites, if any, at the earliest time during drug development. |
| 2. Select the most appropriate biological fluid for drug measurement. |
| 3. Study the relationship between drug concentration and clinical outcome as early as possible during clinical trials. |
| 4. Establish a threshold concentration for effective immunosuppression. |
| 5. Establish an upper limit concentration for reduction of risk for the development of immunotoxicity. |
| 6. Participate in an appropriate quality-assessment program. |

Tests for the inhibition of the target pathways of immunosuppressants are being developed to provide new laboratory evaluation approaches to the assessment of the immunosuppression status of the patient. New approaches to pharmacodynamic monitoring currently under evaluation in transplant patients include calcineurin phosphatase inhibition [53] as an assessment of CsA immunosuppression and inosine monophosphate dehydrogenase inhibition in whole blood as an assessment of mycophenolic acid immunosuppression in the individual patient [54].

Analytical Goals for Immunosuppressive Drug Measurement
For routine therapeutic drug-monitoring purposes, monoclonal antibody immunoassays are most commonly used for CsA measurement, although a substantial number of laboratories are using validated HPLC methods that provide for specific measurement of the parent drug [10]. In a review of the performance characteristics of immunoassays, Oellerich et al. [10] pointed out that because of differences in specificity of the monoclonal antibodies and between-method differences in calibration, CsA measured in the same patient’s blood sample by the three available immunoassays (Abbott TDx, Incstar radioimmunoassay, and Syva–Behring enzyme-multiplied immunoassay technique) can differ by as much as 100%. This degree of discrepancy is especially likely in samples containing high concentrations of cross-reacting CsA metabolites relative to the parent drug. Because CsA metabolites contribute little to overall
immunosuppression, using the least-specific assays in patients with high metabolite concentrations could cause the physician to underdose the patient. There continues to be a pressing need for the development of well-validated analytical methods that meet stringent but realistic criteria for the accurate and reproducible measurement of active drug for CsA [7, 9, 10, 47, 48, 55], and initial experience with tacrolimus measurement shows that this need is as pressing for this immunosuppressive agent [23]. Well-validated analytical methods are also essential in pharmacokinetic studies performed during preclinical and clinical trials of new immunosuppressive drugs [47, 55]. The scientific and, ultimately, the clinical value of such study data depends on rigorous experimental study design such as the RCCT design discussed above and the use of validated analytical methods [55]. A consensus has emerged over the past few years on the desired performance characteristics for acceptable methodology for CsA measurement [9, 10], and such criteria could be used for other immunosuppressive drugs as well. According to these criteria, the recommended between-day precision is a CV of \( \pm 10\% \) at a CsA concentration of \( 50 \mu g/L \) and a CV of \( \pm 5\% \) at a concentration of \( 300 \mu g/L \) [9, 10]. For accuracy, as determined by comparison of a method with a validated HPLC procedure, the recommendation is a slope of the linear regression line of \( \pm 10\% \) from the line of identity, an intercept of \( \pm 15 \mu g/L \), and the \( s_{y|x} \leq 15 \mu g/L \) [9, 10].

References


