
TOXICITY-SPARING PROTOCOLS USING MYCOPHENOLATE MOFETIL IN RENAL TRANSPLANTATION

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INTRODUCTION

The experimental and clinical development of ciclosporin can undoubtedly be regarded as the breakthrough in organ transplantation. In combination with steroids and azathioprine, the famous triple drug regimen, ciclosporin, later on also tacrolimus, greatly reduced the risk of acute rejection. Nevertheless, the long-term results after successful organ transplantation under treatment with calcineurin-inhibitors (CNIs) were and still are disappointing. In fact, it is widely recognized that the long-term use of both CNIs and steroids is associated with serious toxicities that ultimately can impact not only graft but also patient survival.

Major complications with the use of CNIs include nephrotoxicity, atherogenicity, hypertension, hyperlipidemia, and hyperglycemia. Steroids have a broad range of immunosuppressive, anti-inflammatory, and hormonal effects that contribute to their numerous toxicities. These include hypertension, dyslipidemia, weight gain with central obesity, cataract formation and osteoporosis - just to mention a few adverse events out of the whole list known to every transplant clinician.

The introduction of newer agents during the past 15 years such as mycophenolate mofetil (MMF) and the target of rapamycin (TOR) inhibitors, sirolimus and everolimus, with mechanisms of action that are distinct from those of either CNIs or steroids, has facilitated the development of CNI- and steroid sparing therapies. Thus, together with Dr. Flavio Vincenti, I have recently written a review article which highlights studies/trials of MMF-based immunosuppressive regimens in renal transplantation that seek to limit patient exposure to CNIs or steroids (1). In the following, I am going to present some parts of this review by trying, at the end of my talk, to add some theories about possible underlying mechanisms causing a less toxic effect of MMF in contrast to CNIs. And by doing so, I will address some new insights into events of innate immunity because there is obviously an interesting relationship between the beneficial effect of MMF and events of innate immunity.

As outlined in the review article, in principle, MMF-based, toxicity-sparing protocols in renal transplantation can be divided into (I) CNI-sparing/avoidance regimens subdivided into CNI-withdrawal, reduction regimens, CNI-avoidance, and

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delayed introduction - and (II) steroid-sparing/avoidance regimens subdivided into different withdrawal as well as avoidance regimens. Without being complete in regard to this presentation, I will address just a few of those protocols in the following chapters.

MMF-based CNI-sparing/avoidance regimens in renal transplantation

CNI- withdrawal regimens: There were five studies in which patients underwent CNI withdrawal. There were two additional studies in which a subset of patients underwent CNI withdrawal. These studies enrolled patients with stable or declining renal function.

The first trial was a conversion trial performed in patients transplanted at my former institution in Munich in 1996: In this – meanwhile anecdotal - pilot trial, we converted kidney transplanted patients beyond the first year posttransplant - and with stable renal graft function - from CNI – to MMF-monotherapy. Our protocol included a “two-phase”-conversion procedure: after stepwise addition of MMF, the CNI- drug was linearly reduced to zero over a period of 6 weeks. MPA - 12 hour -trough levels were adjusted between 2 – 6 microgram/mL. The conversion procedure was safe: conversion-induced acute rejection episodes occurred in 10,9% - they were mild and could easily be reversed. The long-term results after conversion were satisfactory: the annual graft loss rate was 4,30 % - which, at least in our hands, was improved compared to historical controls. The 5-year – long-term results after conversion to MMF-monotherapy were characterized by a significant improvement of renal allograft function as indicated by a steady decline in serum creatinine and urea values. Similarly, the atherogenic profile: it significantly improved as indicated by a steady decline of triglyceride- and cholesterol values; the blood pressure improved as indicated by a drop of antihypertensive drugs. Strict therapeutic drug monitoring was performed in recognition of the fact that the whole immunosuppressive treatment was depending on MMF alone: Over the 5-year-period, the mean MPA trough –levels showed a slight increase up to 4 microgramm per mL - under a mean daily MMF dose of 1,5 gram.

We were pleased to notice that subsequent international prospective randomized multicentre trials confirmed our original experience.

One of the trials enrolling patients with stable renal function was a multicentre trial involving 170 patients on ciclosporin/steroid maintenance. Following randomization, patients received either MMF/steroids or MMF/ciclosporin/steroids. Significant more patients in the ciclosporin withdrawal group had improved renal function. Withdrawal-induced moderate rejection episodes were observed in 10.6% - a figure nearly identical to that seen in our pilot trial.

A newly published multicentre, international trial evaluated the safety and efficacy of ciclosporin withdrawal in patients at least 6 months posttransplant with deteriorating renal function = the “Creeping Creatinine” Study. In the study group, MMF was introduced followed by gradual withdrawal of ciclosporin over a 6-week period. Renal function, as assessed by serum creatinine, calculated creatinine clearance, and serum urea, improved in the CsA-withdrawal/MMF-treatment group while worsening in the control group with significant differences at both 6 and 12 months. There were no episodes of acute rejection in either group and no differences in graft/patient survival at 12 months.

CNI - AVOIDANCE REGIMENS : CNI-avoidance strategies were employed in eight studies. Since the early posttransplant period is associated with a high risk of acute rejection, seven of the eight studies reported the use of some form of antilymphocyte induction therapy.

Amongst those reports is a recently published trial performed by our Munich group which initiated a study to assess the safety and efficacy of a CNI-free, MMF-based induction/maintenance regimen in elderly recipients of kidneys from elderly deceased donors, with the aim of improving the inferior outcomes often seen in these patients. The immunosuppressive protocol of this prospective pilot trial included an induction treatment which was provided by a combination therapy consisting of MMF, steroids and a short course of ATG. In addition we applied a transient antioxidative cocktail consisting of Vit.C, Vit. E, and acetyl-cysteine intraoperatively up to day 4 postop. The long-term results under MMF-based maintenance look promising: the 5-year – patient and allograft survival was 86,5% and 75,5% , respectively. The 5-year- renal graft survival was even 88% when censored for patient death and non-immune graft loss.

CONCLUSION CNI - sparing/avoiding protocols: Including the data obtained from the other trials not quoted here, one



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may conclude that a variety of MMF-based CNI-sparing/avoiding regimens have demonstrated benefits in preserving or restoring renal function with acceptable levels of risk for mild acute rejection episodes. Careful patient selection and follow-up are important to the success of any CNI-sparing protocol. Because of its non-nephrotoxic and non-atherogenic safety profile, MMF provides an important contribution to the success of CNI-sparing protocols.

MMF-BASED STEROID-SPARING/AVOIDING REGIMENS IN RENAL TRANSPLANTATION

Various steroid-sparing regimens using MMF have been investigated, including dose reduction, withdrawal after initial maintenance period (often involving gradual reduction in dosage prior to discontinuation), early (rapid) withdrawal, or complete avoidance of steroids from the time of transplant. Not all those regimens are quoted here. However, representative for all those trials is the most recent, most innovative and interesting approach: the complete avoidance of steroids! Four studies evaluating complete steroid-avoidance regimens can meanwhile be identified in the literature. In the largest study out of three non-randomized trials, 100 patients received antithymocyte globulin induction and MMF/CsA maintenance therapy. These patients had a 13% incidence of acute rejection with a 4-year graft/patient survival of 82% and 99%, respectively. A large, randomized multicentre trial of steroid avoidance with the use of daclizumab induction was recently completed and reported. Patients received either tacrolimus, MMF and steroids, or tacrolimus, MMF, and daclizumab. At 6 months, there were no significant differences in patient/graft failure, or the incidence of acute rejection, despite the fact that 89% of patients completing the study in the daclizumab group remained free of steroids.

CONCLUSION: BENEFITS AND RISKS OF STEROID-SPARING REGIMENS: Including all the other data from studies not mentioned here, one can conclude that steroid-sparing maintenance regimens were successful in most patients who received them, with the majority remaining on steroid-free therapy at the end of the assessment period. Few studies demonstrated significant benefits of steroid-sparing regimens in adult patients within a generally short follow-up period although reductions in blood pressure and hyperlipidemia are potential benefits. In pediatric patients, however, steroid-sparing maintenance was not only safe but

resulted in pronounced improvements in growth and body morphology. Thus, steroid reduction or avoidance is highly desirable for pediatric patients. There did not appear to be any differences in the safety of early withdrawal/avoidance regimens and late withdrawal in the reviewed literature, suggesting that earlier interventions may be a preferred strategy, with rapid withdrawal or complete avoidance of steroids in those patients deemed at high risk for steroid-related adverse events.

THEORETICAL BACKGROUND OF THE BENEFICIAL EFFECT OF MMF SEEN IN CLINICAL TRIALS

The theoretical background of the beneficial effect of MMF observed in many clinical trials appears to be related to the fact that this non-organ-toxic drug – in contrast to CNIs – does not activate cells of innate immunity such as vascular cells – because it does not cause tissue injury as the crucial factor for innate immunity activation.

And in this context, the findings are of importance indicating that there are different effects of CNIs and IMPDH inhibitors such as MMF on the generation of the toxic reactive oxygen species causing tissue injury. In fact, Cyclosporin generates free oxygen radicals whereas MMF even reduces its generation. Mechanisms of Cyclosporin-induced reactive oxygen species – according to reports of the literature – include the generation of superoxide anions as well as free radical nitric oxide. Free superoxide radicals appear to be generated during the metabolism of Cyclosporin within the cytochrome P450 enzyme system – but this interpretation is still elusive. There seem to be some unknown sources of ROS-generation not yet detected so far. Nitric oxide is generated during the reaction L-arginine to citrulline which is catalysed by nitric oxide synthetase (= NOS). Cyclosporin has been shown to upregulate endothelial NOS. Superoxide anions and nitric oxide, then, form the very toxic peroxynitrite.

In contrast are IMPDH-inhibiting drugs such as MMF: Instead of up-regulation of oxidative stress, they may even diminish it: Mycophenolic acid is able to inhibit the action of iNOS, and by this, inhibits NO-generation – followed by inhibition of formation of peroxynitrite. The mechanism behind is that NOS needs a cofactor for reaching full activity which is BH4- a tetrahydrobiopterin. This protein needs GTP arising from IMP. MPA inhibits the generation of GTP which leads to inhibition of BH4-formation – associated with inactivity of NOS.

New notions on the role of innate immunity in organ transplantation hold that any allograft-injury, including drug-mediated injury, activates the innate immune system resulting in acute allograft rejection and chronic renal allograft dysfunction. Ciclosporin, via induction of oxidative stress and renal injury, may activate cells of innate immunity. Activated dendritic cells would be able to initiate the alloimmune response, however, due to its strong immunosuppressive effect, the drug inhibits subsequent events of adaptive alloimmune response. Nevertheless, the nephrotoxic drug still may contribute to alloatherosclerosis and allobfibrosis leading to allograft dysfunction. In fact, this scenario, the activation of innate immunity by ciclosporin, has been recently experimentally confirmed by a Korean group performing studies in rats: This study revealed the up-regulation of Toll-like receptors (TLR2 and TLR4) expression and heat shock protein 70 expression in rat kidneys under exposure to ciclosporin.

CONCLUSION

Long-term use of both CNIs and steroids is associated with adverse effects in renal transplant patients. Immunosuppressive regimens that reduce or eliminate exposure to CNIs and steroids are now feasible with the

introduction of newer immunosuppressive agents such as MMF. Data are emerging that MMF “toxic-sparing” strategies are safe and associated with reduction of toxicities that accompany the use of CNIs and steroids.

In particular, the beneficial effect of MMF on long-term allograft survival may be due not only to its immunosuppressive potency, its lack of organ toxicity but also to its capability to diminish oxidative injury. According to new insights into mechanisms of innate immunity, lack of organ toxicity prevents MMF from activation of cells of the innate immune system (dendritic cells, vascular cells) and, thus, may ameliorate and reduce this innate system’s secondary consequences such as initiation / maintenance of alloimmune responsiveness (e.g. subclinically ongoing rejection episodes); contribution to the development of alloatherosclerosis (mediating chronic dysfunction of donor organs), and to the development of auto-atherosclerosis (contributing to cerebro-cardiovascular accidents of recipients).

REFERENCE

Land W, Vincenti F. Toxicity-sparing protocols using mycophenolate mofetil in renal transplantation. *Transplantation* 2005; 80 (2S): S1-S14.

*I don't know what your destiny
will be, but one thing I know: the
only ones among you who will
be really happy are those who
will have sought and found how
to serve.*

- ALBERT SCHWEITZER

