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Monitoring immunosuppression after liver transplantation : development of individualized Bayesian limited sampling monitoring

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CHAPTER 1

INTRODUCTION

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INTRODUCTION

The first experimental attempts of liver transplantation on dogs were in 1955 by Welch[1]. In 1963 Thomas E. Starzl and colleagues started human liver transplantation[2]. Like the first two of these operations in the Netherlands in Leiden and Arnhem in 1966 and 1968 respectively, these were unsuccesfull auxiliary liver transplantations. Operation technique and medication apparently were not yet ready. After a self-imposed moratorium and more animal experiments Thomas E. Starzl in Denver and also Sir Roy Calne in Cambridge started human orthotopic liver transplantation (OLT) in 1978, and in the Netherlands the fifth center worldwide started in Groningen in 1979 (Gips, Kootstra and Krom). In 1983 at a National Institutes of Health Consensus Development Conference it was decided that liver transplantation was no longer experimental and deserved broader application in clinical practice[3]. Nowadays thousands of OLTs have been performed successfully. The one-year survival is 90% and the 5-year survival over 80% in many centers. This is due to many factors like improved operative technique, better prevention, recognition and treatment of complications, and improved immunosuppression.
The first use of immunosuppressive agents in OLT was in 1966 with a prednisolone and azathioprine schedule derived from the successful kidney transplantations[4]. The breakthrough of the use of immunosuppressive agents in OLT was in 1980, the development of cyclosporine, a calcineurin-inhibitor. Cyclosporine was effective in the prevention of rejection and there was an increase in the survival rate after OLT[5-9]. Later on, other immunosuppressants like tacrolimus (FK-506, another calcineurin inhibitor) and mycophenolate mofetil (MMF) were introduced for prevention of graft-loss due to rejection. With the success of these agents the focus is now shifting towards reduction of side-effects from these drugs, including renal insufficiency from calcineurin inhibitors. Therapeutic drug monitoring (TDM) is an important tool for achieving these goals. This thesis focuses on TDM of cyclosporine, tacrolimus and mycophenolate mofetil after OLT.

Cyclosporine

The drug cyclosporine (Neoral®) is a cyclic polypeptide immunosuppressant agent consisting of 11 amino acids. It is produced as a metabolite by the fungus species Beauveria nivea. The effectiveness of cyclosporine results from specific and reversible inhibition of immunocompetent lymphocytes in the G0- and G1-phase of the cell cycle. T-lymphocytes are preferentially inhibited. The T-helper cell is the main target, although the T-suppressor cell may also be suppressed. Cyclosporine also inhibits lymphokine production and release including interleukin-2[10] (Figure 1).

Tacrolimus

Tacrolimus (Prograf®), previously known as FK506, is a macrolide immunosuppressant produced by *Streptomyces tsukubaensis*. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (i.e. immunosuppression)[11] (Figure 1).

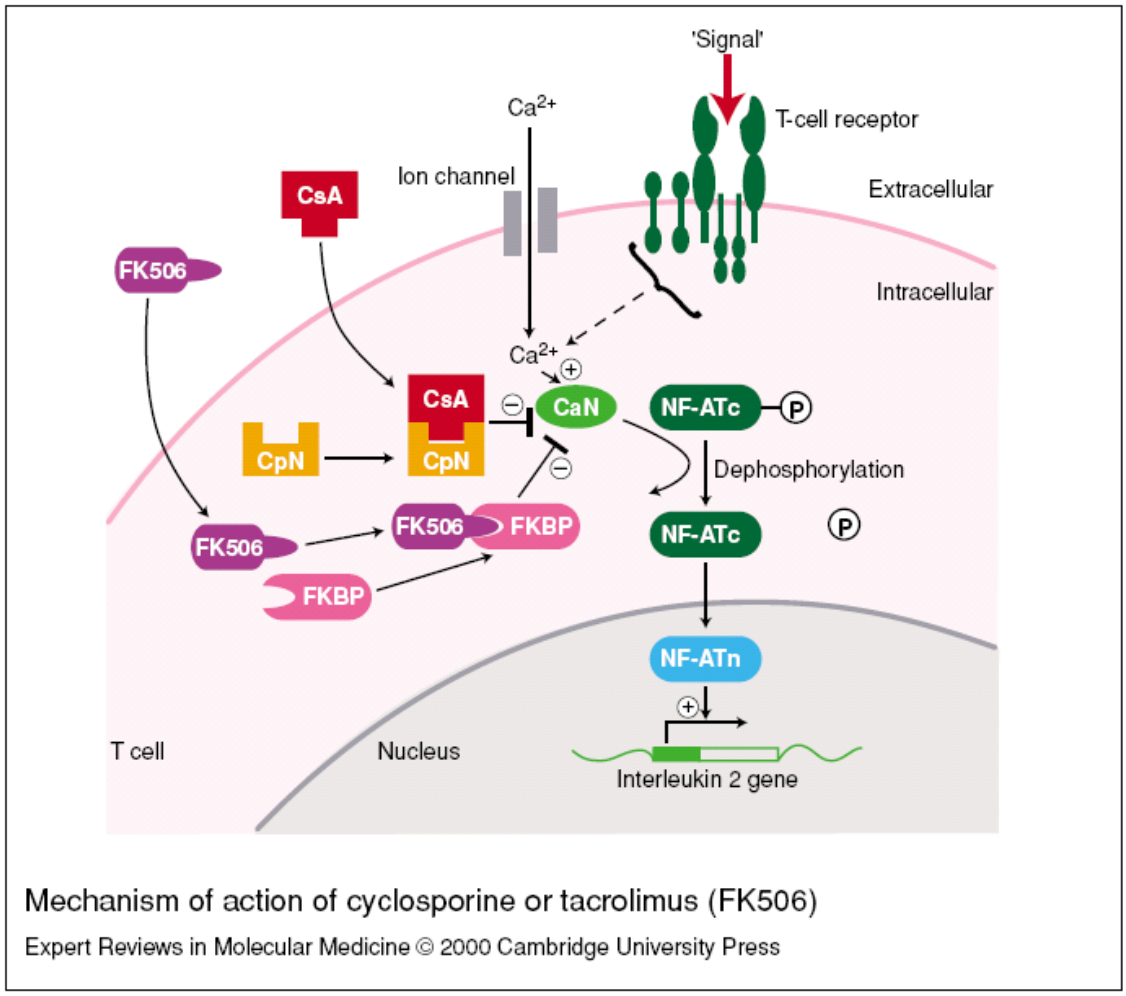

Figure 1: Mechanism of action of cyclosporine and tacrolimus

Mycophenolate mofetil

Mycopheonale mofetil is the 2-morpholinoethyl ester of mycophenolic acid (MPA), an immunosuppressive agent, which is an inosine monophosphate dehydrogenase (IMPDH) inhibitor. Mycophenolate mofetil is rapidly absorbed following oral administration and hydrolyzed to form MPA, which is the active metabolite. MPA is a potent, selective, uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Because T- and B-lymphocytes are critically dependent for their proliferation on de novo synthesis of purines, whereas other cell types can utilize salvage pathways, MPA has potent cytostatic effects on lymphocytes. MPA inhibits proliferative responses of T- and B-lymphocytes to both mitogenic and allospecific stimulation. Addition of guanosine or deoxyguanosine reverses the cytostatic effects of MPA on lymphocytes. MPA also suppresses antibody formation by B-lymphocytes. MPA prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion to endothelial cells and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection. Mycophenolate mofetil did not inhibit early events in the activation of human peripheral blood mononuclear cells, such as the production of interleukin-1 (IL-1) and interleukin-2 (IL-2), but did block the coupling of these events to DNA synthesis and proliferation[12] (Figure 2).

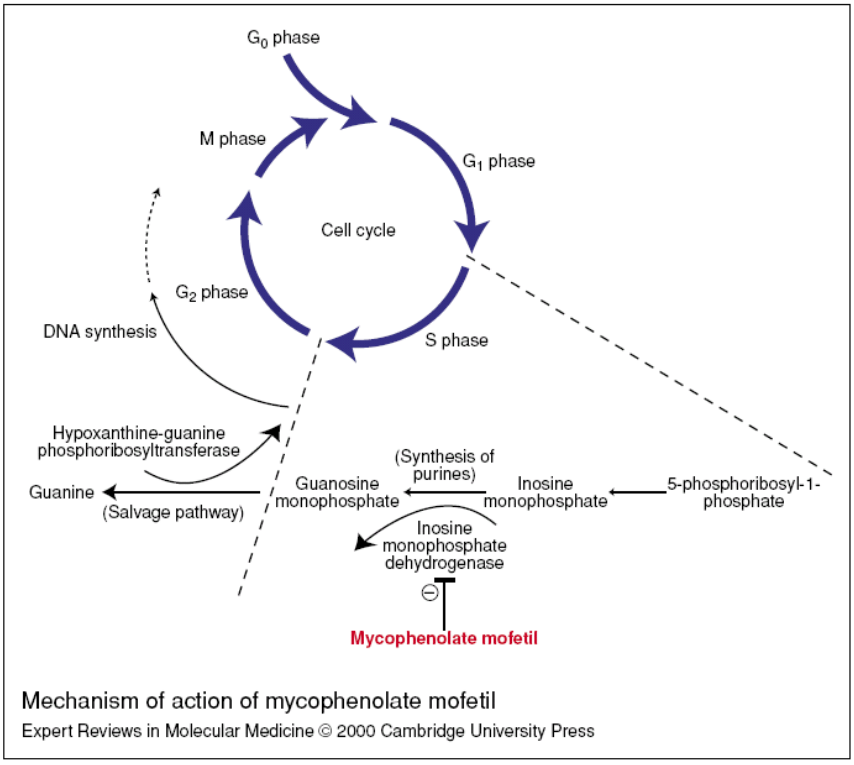

Figure 2: Mechanism of action of mycophenolate mofetil

Therapeutic drug monitoring

Calcineurin inhibitors (cyclosporine and tacrolimus) are characterized by a narrow therapeutic window. Underdosing may lead to acute or chronic rejection of the graft, while overdosing may lead to adverse effects, like elevated blood pressure and nephrotoxicity. Therefore accurate dosing of these drugs is warranted.
When using therapeutic drug monitoring (TDM) dosing is based on measured drug-concentrations in blood. Dependent on these concentrations the dose is adjusted. Especially for medication with a narrow therapeutic range the use of TDM is very useful. This is exactly the reason why in the past decades many studies have been performed to develop different strategies for TDM in organ transplantation.

Trough concentration (C0) monitoring

For many years trough concentration or C0 monitoring was generally accepted as the best way of monitoring cyclosporine and tacrolimus. This means that dose and possible dose adjustments were based on the blood concentration sample just before taking the medication. Both cyclosporine and tacrolimus are mostly dosed twice daily, which means that a predose-level (C0) is taken approximately 12 hours after the last dose. C0-monitoring was proven to be effective in reducing rejections and adverse events. Later, the question arised whether C0-monitoring was the optimal way of therapeutic drug monitoring, particularly for cyclosporine. Studies showed that the correlation of C0 with the area under the concentration time curve for 12 hours (AUC0-12) was poor and that other time sampling points may better reflect systemic exposure of cyclosporine for a dosing interval. Subsequently, a new widely introduced strategy for cyclosporine was C2-monitoring.
For tacrolimus nowadays C0-monitoring is still the common strategy in most clinics.

Fixed dose regimens

In contrast to the calcineurin inhibitors cyclosporine and tacrolimus, there is no consensus on the need for therapeutic drug monitoring of mycophenolate mofetil (MMF). Most centers adhere to fixed dose regimens, which means that dosing is not based on blood concentrations or other clinical properties like weight, co-medication or liver and kidney function. Recently, different strategies were studied including C0-monitoring, but there seemed to be a weak correlation between C0 and AUC.

Limited sampling strategies

The exposition to a drug is determined by the ‘gold standard AUC’. Which is approximated by taking blood samples every hour and integrating these data with the ‘trapezoidal rule’. Next to (fixed) single time points as a basis for therapeutic drug monitoring of immunosuppressive drugs also limited sampling strategies have been

developed in the past decade. This means that multiple blood sampling time points are used in a formula or model as a surrogates for the 'gold standard' AUC0-12h. Most of these strategies are using limited sampling formulas (LSF algorithms). These have the disadvantage that the blood sampling needs to be performed exactly on time, which is difficult in an outpatient clinic.

Modeling based on Bayesian estimation

Few studies have been performed on the development of limited sampling models (LSM) based on Bayesian estimation, a statistical method successfully used in pharmacy but also other fields of medicine. The advantage of these models is that they are flexible, accurate and easy to apply in practice without the need to take blood samples exactly on time. As long as the sampling time is noted, these limited sampling models (LSMs) are accurate, in contrast to the rigid limited sampling formulas (LSFs), if blood is not drawn exactly on time.

Aim of the thesis

In this thesis we try to optimize the therapeutic drug monitoring of cyclosporine, tacrolimus and mycophenolate mofetil in liver transplant patients with limited sampling strategies and modelling, using Bayesian estimation.

Recent literature from studies -more performed in kidney than liver transplantation- suggested that a new way of monitoring cyclosporine in organ transplantation patients (C2-monitoring) better predicted the systemic exposure to the drug over the first 12 hours after dosing than C0-monitoring did, which may lead to improved clinical outcome[13-26]. C2 was then recommended for monitoring cyclosporine. Due to this recommendation in **chapter 2** we switched our stable patients more than 6 months after OLT from C0-monitoring towards C2-monitoring and investigated the influence of this switch on factors as dose, creatinine clearance (CRCL), blood pressure and freedom from rejection and the relationships of C0 and C2 with the gold standard AUC0-12h.

In **chapter 3** we were looking for even better methods for monitoring cyclosporine[27]. We developed and validated an easy to use, accurate and flexible individualized Bayesian population-pharmacokinetic (POP-PK) limited sampling model (LSM) integrating all available information, without the need for fixed blood sampling time points. Different limited sampling models were tested and the correlation of these models with the 'gold standard' AUC0-12h was calculated, in order to predict the systemic exposure of cyclosporine very precisely with a limited number of blood samples.

The limited sampling model with time points 0 + 1 + 2 + 3h was then introduced into our clinic[28]. In **chapter 4** we evaluated the patients who were previously switched from C0 to C2 and now switched to LSM 0,1,2,3h after using this model in our clinic for over

18 months. This allowed us to investigate the feasibility of implementation of LSM in practice, and the potential effects on factors as dose, renal function and rejection rate of the three monitoring strategies, and also inter- and intrapatient variability in pharmacokinetics of cyclosporine using LSM. We determined the required precision of the method used and a new target range for cyclosporine AUC was calculated.

Another frequently used calcineurin inhibitor, tacrolimus, is just as cyclosporine characterized by a narrow therapeutic range. This underlines the need of accurate monitoring to prevent rejection and adverse events for this drug as well. The monitoring of tacrolimus is still based on C0-monitoring in most centres. Recent data showed that other blood sampling time points than C0 may better reflect systemic exposure to tacrolimus[29-32]. In **chapter 5** we examined which single time point or combination of time points best reflect systemic exposure to tacrolimus, estimating the area under the concentration time curve. We calculated limited sampling formulas and developed a new and flexible limited sampling model for monitoring tacrolimus concentration which is easy to apply in the outpatient clinic, as we did earlier for cyclosporine[28].

Mycophenolate mofetil (MMF) is increasingly used after OLT, since in contrast to calcineurin inhibitors (CNI) like cyclosporine and tacrolimus MMF is not nephrotoxic. It may allow CNI reduction or discontinuation, resulting in improvement or stabilization of renal function[33]. Most clinics adhere to a fixed dose of MMF, not based on any individual patient or population characteristics[34]. Recent studies with conflicting results and limitations have been performed to explore current evidence and clinical relevance of TDM (C0 and limited sampling strategies) of MMF[35-40]. Limited information on this is available in liver transplant patients[41-42]. In **chapter 6** we described the pharmacokinetic behaviour of MMF in stable liver transplant patients and looked at possible relationships of albumin concentration, creatinine clearance and co-medication (especially calcineurin inhibitors) with MPA clearance, the active metabolite of MMF. Furthermore we investigated the correlation of C0 with AUC0-12h and possible interpatient variability. Finally we developed different limited sampling models for implementation of therapeutic drug monitoring of MMF in liver transplant patients, with special attention to kidney function in patient selection.

In **chapter 7** we summarize the results of our studies and we discuss the possible role of our findings for clinical practice, now and in the future.

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