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Drug target molecules to guide immunosuppression

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ABSTRACT

The individual and interindividual variability of response to immunosuppressants combined with the prevailing concept of lifelong immunosuppression following any organ transplantation motivates the search for methods to further individualize such therapy. Traditional therapeutic drug monitoring, adapting dose according to concentrations in blood, targets the pharmacokinetic variability. It has been increasingly recognized, however, that there is also a considerable variability in the response to a given concentration. Attempts to overcome this variability in response include the efforts to identify relevant targets and methods for pharmacodynamic monitoring. For several of the currently used immunosuppressants there is experimental data suggesting markers that are relevant as indicators for individual monitoring of the effects of these drugs. There are also some clinical data to support these approaches; however what is generally missing, are studies that in a prospective manner demonstrates the benefits and effects on outcome. The monitoring of antithymocyte globulin by lymphocyte subset counts is actually the only well established example of pharmacodynamic monitoring. For drugs such as MPA and mTOR inhibitors, there are candidates such as IMPDH activity expression and p70SK6 phosphorylation status, respectively. The monitoring of CNIs using assays for NFAT RGE, either alone or combined with concentration measurements, is already well documented. Even here, some further investigations relating to the categories of organ transplant, combination of immunosuppressants etc. will be requested. Although some further standardization of the assay is warranted and there is a need for specific recommendations of target levels and how to adjust dose, the NFAT RGE approach to pharmacodynamic monitoring of CNIs may be close to implementation in clinical routine.

1. Introduction

Although the incidence of rejection episodes after organ transplantation has been reduced to a low level in recent years, there is still room for improvement of the immunosuppressive therapy. For most organ transplant recipients, the use of immunosuppressants needs to be continued lifelong. These medications are associated with drug specific adverse effects such as diabetes and osteoporosis plus the side effects of immunosuppression which include increased risk of infections, malignancies and reduced life expectancy also due to increased risk factors for cardiovascular disease. On this background the search for a better optimization and individualization of immunosuppressive therapy is warranted. Current therapeutic drug monitoring is aiming to compensate for pharmacokinetic variability. The inter- and intraindividual variation in response – i.e. pharmacodynamic variability, may be even larger. New knowledge about the mechanisms of action of immunosuppressive drugs at the molecular level may provide opportunities for a more advanced individualization of treatment. In this review the targets for each group of immunosuppressants and the status for their potential as biomarkers to guide individual dosing are summarized.

2. Calcineurin inhibitors (CNI)

2.1. Tacrolimus and cyclosporine

Tacrolimus (TAC) and cyclosporine A (CsA) are the calcineurin inhibitors frequently used in organ transplantation. In recent years TAC has to a large degree replaced CsA as the drug of first choice in this group. The targets for these drugs are mainly the same, but there is also some dissimilarity that could provide a basis for differentiation in the pharmacodynamic (PD) monitoring approach.

2.1.1. Calcineurin and immunophilin

The activity of the phosphatase calcineurin increases in response to activation of T-cells. Calcineurin dephosphorylates the cytoplasmic nuclear factor of activated T-cells (NFAT), leading to increased translocation of the latter into the nucleus. The NFAT binds to promoter sites of DNA encoding interleukin-2 (IL-2) and several other cytokines that

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will enhance and maintain the immune response. When CNIs enter the T-cells, they form complexes with their respective binding proteins and associate with calcineurin, thus reducing the phosphatase activity versus NFAT and thereby inhibiting this pathway of the immune response. The major binding protein of CsA is the immunophilin cyclophilin A while TAC is mainly associated with the immunophilin called FK-binding protein FKBP12. The affinities for calcineurin for these proteins are enhanced as they combine with CsA and TAC, respectively.

Early in the nineties it was established that calcineurin was a key enzyme in T-cell signaling, and that the inhibition of the calcineurin-calmodulin complex was an important mechanism for the inhibition of the T-cell response by CsA and TAC. The group of Halloran et al. investigated the relation between CsA concentrations and calcineurin inhibition [1]. Following these observations, several studies were performed in organ transplant recipients, assessing the calcineurin phosphatase activity during CsA treatment [2,3]. These studies have indicated that assessment of calcineurin activity could be useful as a supplement to the current TDM in the individualization of CNI therapy. As presented so far, the assays are quite complex and laborious [4] –which may be one explanation for the lack of larger studies that are needed to confirm its potential usefulness.

2.1.2. Cytokines

Dephosphorylation of NFAT is the immediate downstream effect of calcineurin, hence the effect of CNIs is inhibition of this dephosphorylation. Therefore, some measurement of NFAT effect presents itself as a potential pharmacodynamic marker specific for the CNIs. Cytokines such as IL-2 and interferon (IFN)-γ in serum or following mitogen activation in whole blood have been assessed [5], but there are problems related to short half-lives and multiple factors influencing their levels other than the calcineurin inhibition. A couple of studies in liver transplant recipients have related increased IL-2 levels to rejection [6,7]. A study of liver transplant recipients on a TAC based regimen demonstrated that patients with less inhibition than 40% of the biomarkers during the first week post-transplant, experienced a biopsy proven rejection. The significant biomarkers were soluble IFN-γ measured by ELISA and the expression of IFN-γ and IL-2 in CD8 + cells, assessed by flow cytometry. Moreover, in this study IFN-γ inhibition less than 15% in the first week was associated with the more severe rejections, and high IFN-γ expression in CD8 + cells pretransplant also predicted rejection [8]. The expression of these cytokines are also components of the assay that is described in the following paragraph.

2.1.3. NFAT residual gene expression

In 2006 Sommerer et al. reported the use of an assay that estimated the residual expression of three NFAT-regulated cytokines (NFAT RGE). Among the 133 stable renal allograft recipients on CsA-based immunosuppression, the measured NFAT RGE was significantly lower both in patients with infections and in those that developed malignancies, respectively. By the combination of these data, a critical cut off at 15% NFAT RGE was identified. Importantly, this was in contrast to the CsA trough or two hour whole blood concentrations, which did not correlate with these outcomes [9]. The assay that was introduced in this study employed the principles from previous investigations suggesting that the combination of more than one relevant cytokine mRNA expression could be used for pharmacodynamic monitoring of CsA and tacrolimus [10,11]. The cytokines included in the study by Sommerer, was IL-2, IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF); i.e. their respective genes *IL2*, *IFNG* and *CSF2*. The quantification of cytokines mRNA is performed by quantitative PCR (qPCR) following stimulation of whole blood samples using PMA and ionomycin, including calculation of the expression relative to reference genes. This study also introduced a concept for normalization by performing the measurements at two time points, predose and two hours after CsA intake, and then calculating the NFAT RGE as the percentage ratio between the two.

Following these early investigations, several studies have employed the NFAT RGE method to investigate the potential for optimization of CNI individualization. The majority of these studies originate from the same group. In a successive series of publications they have demonstrated that in renal allograft recipients receiving CsA, the NFAT RGE correlates with occurrence of adverse effects such as CMV viremias, infections, non-melanoma skin malignancies and gingival overgrowth [9,12–16]. In the elderly patients there was also a correlation to renal function [15]. Furthermore, a dose reduction study was performed in 20 stable renal transplant recipients with matching controls. The CsA dose reductions were paralleled by increasing NFAT RGE, a stable estimated glomerular filtration rate (eGFR) compared to deterioration in the controls and a reduction in blood pressure. In one patient the dose reduction lead to a biopsy proven rejection, following a NFAT RGE increase to 47% -while for the other nineteen patients the range was 7–32% [17]. The correlation of NFAT RGE with infections was even reported for cardiac allograft recipients [18]. Another study including 20 de novo and 20 stable liver transplant recipients reported NFAT RGE around 16–17% and a correlation with infections plus a correlation between NFAT RGE and CsA concentration at two hours, but not at trough [19].

With respect to TAC, there are studies in both kidney and liver transplants that have reported somewhat similar findings as for CsA [20–24]. However, important differences are observed between the two CNIs. In a study of stable pediatric liver transplant recipients, NFAT RGE correlated with the occurrence of infections in the CsA treated but not with TAC. An observation repeated in several studies, is that the NFAT RGE is not influenced to the same degree by TAC compared to CsA, in the currently accepted standard dosages which are now lower than in the early trials [21]. Still, as documented in several trials, TAC is efficient in preventing rejections at these dosages, and therefore it is suggested that for TAC there are other mechanisms of actions that contribute to its effect. This is highlighted in two recent publications. Whereas the numbers were small, the NFAT RGE was higher in the patients experiencing rejections, for the CsA-treated (39% vs 11%) as well as the TAC-treated (48% vs 18%) [25]. In the second study, NFAT RGE in liver transplant recipients with ongoing CMV infections were compared to controls without infection. Although both in the CsA (30% vs 44%) and the TAC group (68% vs 84%) the NFAT RGE for CMV infected vs controls was marginally not significant, a trend was observed, and in both groups the difference specifically for *IFNG* residual expression was significant [23].

A general finding in the studies mentioned above, is that when there was a trend or a significant correlation between NFAT RGE and outcome, mostly a similar association was not regularly seen for the measured CNI trough concentrations, but to a larger degree with the peak concentrations (CsA 2 h and TAC 1.5 h). Current recommendations are based on studies in which the CNI was combined with glucocorticoids, mostly also mycophenolate and an IL-2R antibody, but without T-cell depleting induction. The studies indicate that including NFAT RGE as a means for individualized dosing may provide improved long term results, exemplified by the successful dose reductions in patients with the lowest NFAT RGE. To define a generally recommended target RGE, these studies also have in common that with the currently most successful dosing regimens for TAC, the average NFAT RGE is higher than with CsA. A tentative target for NFAT RGE in CsA based immunosuppression may then be around 15–30%, while for TAC it may be higher. Further studies are needed to define these targets. Indeed there are ongoing prospective trials in lung and renal allograft recipients respectively, in which the intervention is to adjust the TAC dose in order to obtain NFAT RGE in the range 15–80% (ClinTrials.gov id NCT01771705).

For results to be comparable across studies, some standardization of the NFAT RGE assay is mandatory, as has also been pointed out in recent reviews [26,27]. As of today, the NFAT RGE assay is in daily routine in a limited number of centers. Some established critical principles for performing the assay can be summarized as follows: Expression of the