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Recent advances in therapeutic drug monitoring of immunosuppressive drugs

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ABSTRACT

The transplantation of a solid organ is always followed by a lifelong immunosuppressive therapy to guarantee the survival of the organ in the recipient. Immunosuppressive drugs have to be applied in order to preserve the graft and at last the patient's life. These drugs are strongly recommended for therapeutic drug monitoring (TDM) in order to adjust the adequate dose for each patient to avoid rejection or adverse effects of the therapy. This finely tuned therapy would not be possible without the proper analytical tools and techniques. Either chromatography methods or immunoassays based on e.g. fluorescence or colorimetric detection principles are used in practice.

All of them have to cope with the challenging, individually differing matrix whole blood. It is difficult to reach the relevant levels of quantification.

The focus of this review is on the explanation, comparison and future outlook of the current analytical techniques for TDM of immunosuppressants.

Contents

1. Introduction

For a long time, organ failure was the immediate death sentence of the patient. Nowadays, organ transplantations of livers, kidneys and hearts save many lives each year. Unfortunately, there are not enough organs to save each patient's life. The available organs need to be used as efficient as possible and the function has to be ensured continuously. It is essential to perform an immunosuppressive therapy to avoid rejection of the transplanted organ [1]. The therapeutic index of the used immunosuppressive drugs is extremely narrow. Consequently, a lifelong monitoring of blood levels needs to be done.

Abbreviations: CsA, cyclosporine A; DBS, dried blood spots; FKBP12, FK506 (tacrolimus) binding protein 12; ISD, immunosuppressive drug; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; MPA, mycophenolic acid; mTOR, mammalian/mechanistic target of rapamycin; POCT, point-of-care testing; PSI, proliferation signal inhibitors; SPE, solid phase extraction; TBS, Tris buffered saline; TDM, therapeutic drug monitoring; UHPLC (UPLC), ultra high performance liquid chromatography.

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Generally, there are different approaches of monitoring laboratory parameters. The classic way is to draw a sample once or twice a day and analyze it at the central laboratory of the hospital. Several hours pass until the physician has the result in his or her hands. A faster decision needs to be taken, if the patient is in critical conditions, e.g. multimorbid or early after transplantation. As the most recent tool, Point-of-care testing (POCT) devices, can conduct semi-continuous measurements at the bedside of the patient, favored with regenerable biosensors [2]. One measurement is conducted at the bedside within minutes, making a faster on-site decision of physicians possible. A real-time measurement, as performed in online blood gas analysis [3], is not practicable for Therapeutic drug monitoring and not essentially needed.

Currently, immunosuppressive drugs are monitored at central laboratories by chromatography methods like UPLC-MS/MS or LC-MS/MS and immunoassays based on e.g. fluorescence or colorimetric detection principles. The measurements are mainly performed once or twice a day, right before the administration of a new dosage. In critical conditions of the patient, measurements in shorter time intervals are needed to be done to get clear information about pharmacokinetics of the applied drug. The sample matrix is whole blood in the majority of cases. The high and individually fluctuating content of proteins and products of metabolism poses a challenge for all analytical methods. It often has to be facilitated by a preparative precipitation step to perform reliable measurements.

Nevertheless, it is difficult to reach the relevant levels of quantification, especially in some cases when the assay needs to differentiate between the drug, active and inactive metabolites of it. Additionally, the required drug levels are being lowered more and more to avoid long term toxicity of the applied immunosuppressants. The focus of this review is on the multifaceted realization of therapeutic drug monitoring of immunosuppressive drugs. It points out how current analytical techniques, being used in practice, work and how they perform. Furthermore a glance on possible future developments is given, as they might overcome the obstacles of the currently used techniques.

2. Therapeutic drug monitoring (TDM)

Therapeutic drug monitoring (TDM) describes the measurement of drug concentrations in blood and is done if there is no clinical marker or parameter, which can easily be monitored. Therefore, a relationship between dose and blood concentration and between blood concentration and therapeutic effect of the drug has to exist [4,5]. Criteria for a drug to be suitable for TDM [4] are:

- Relationship between concentration and effect
- Narrow therapeutic index
- Inter-individual pharmacokinetic variability: poor relationship between dose and drug response
- Pharmacological response should be difficult to assess or distinguish from adverse effects

The goal is to individualize therapy of difficult-to-manage medication, assuming knowledge about pharmacokinetics and pharmacodynamics and at last to optimize clinical outcome. The list of drugs, which are recommended for therapeutic drug monitoring, is further growing. Important therapeutic groups are anticonvulsants, cardio active drugs, some antibiotics, psychotropics, immunosuppressants, cytotoxics and hormones [6].

The monitoring of immunosuppressive drugs (ISD) after organ transplantation is strongly recommended [7] for several reasons. The narrow therapeutic index of the drugs leads to severe adverse effects, e.g. nephrotoxicity, if the drug is overdosed whilst underdosage can result in graft rejection. Another characteristic of immunosuppressive drugs is the high degree of inter-individual differences in bioavailability. Pharmacokinetics like differences in absorption, distribution, metabolism and elimination have to be considered, because they can be influenced by gender, age, genetical polymorphisms and renal or biliary insufficiency of the patient. Drug-interactions and inflammation can influence pharmacokinetics additionally [8]. Immunosuppressant drug interactions require prompt action: Identification and intensified TDM with adequate dosing responses [9].

3. Immunosuppressants and their mechanism of action

In 1949 Hench et al. discovered the immunosuppressive effect of Cortisol [10], followed by cyclosporine A (CsA) in 1976 [11], sirolimus (rapamycin) in 1977 [12], tacrolimus in 1987 [13] and mycophenolic acid (MPA) in 1991 [14], to name the most important immunosuppressive drugs [15]. The currently most commonly used immunosuppressive drugs are listed in Table 1.

Tacrolimus and CsA are calcineurin inhibitors (CIs). They bind to immunophilins and block the effect of calcineurin. This results in a reduced production of IL-2 and reduced proliferation of T lymphocytes, which are crucial for immune response. CsA binds to the immunophilin cyclophilin, whereas tacrolimus binds to the immunophilin FKBP12. The complex of tacrolimus and FKBP12 inhibits the calcium dependent protein phosphatase activity of calcineurin-calmodulin complex, inhibiting both T lymphocyte signal transduction and IL-2 transcription by blocking TNFα gene transcription [16]. Calcineurin inhibitors induce vasoconstriction in the kidney, which leads in combination with the overexpression of transforming growth factor to interstitial fibrosis [17]. To avoid these side effects, the calcineurin inhibitor concentration is lowered by combining them with MPA or sirolimus.

The group of antiproliferative drugs or proliferation signal inhibitors (PSIs) consists of sirolimus (rapamycin) and everolimus. They

Table 1
Immunosuppressive drugs and their mechanisms of action. Their target in the body and whether TDM is advised or not is shown in the table. If yes is in brackets in the TDM column it means that TDM influences the outcome of some patients in a positive way but is not yet mandatory

Immunosuppressive Drug	Mechanism of action (target)	TDM
Cyclosporine A	Kinase and phosphatase inhibitors (Calcineurin, JNK/p38 kinase)	yes
Tacrolimus	Kinase and phosphatase inhibitors (Calcineurin, JNK/p38 kinase)	yes
Sirolimus (Rapamycin)	Kinase and phosphatase inhibitors (Cyclin kinase cascade)	yes
Everolimus	Kinase and phosphatase inhibitors (Cyclin kinase cascade)	yes
Mycophenolic acid/ Mycophenolate mofetil (prodrug)	Inhibition of purine synthesis (inosine-5′-monophosphate dehydrogenase)	(yes)
Methotrexate	Inhibition of purine and pyrimidine synthesis (Thymidylate synthase, several enzymes)	yes
Glucocorticoides (Prednisolone)	Regulation of gene expression (Glucocorticoid receptors)	no
Basiliximab (antibody)	CD 25, IL2-R-α-chain	no
Anti-thymocyte globulin (antibodies)	Antibodies against human T lymphocytes	no
6-mercaptopurine/ Azathioprine (prodrug)	6-mercaptopurine is metabolized to thioguanine nucleotids; cytotoxicity resulting in immunosuppression and inhibition of DNA synthesis (polymorphisms or activity of thiopurine methyltransferase are checked before administration)	(yes)
Belatacept (fusion protein)	Inhibition of CD28 mediated costimulation of t cells (CD 80 and CD 86 on antigen presenting cells)	no