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MONITORING TACROLIMUS WHOLE BLOOD CONCENTRATIONS

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Tacrolimus (*TAC*) is the primary drug for most immunosuppressive therapy regimens. It has a narrow therapeutic index, meaning that insufficient dose can lead to graft and tissue rejection, while overdose can lead to increased risk of infections, toxicity, and cancerous tumors in organ transplant recipients. *TAC* belongs to a group of calcineurin inhibitors inhibiting T-cell activation. The use of *TAC* requires regular clinical observation of recipients and laboratory monitoring of the drug concentrations in the blood. This is to ensure correct dosage of the drug and to limit the potential risk of harmful side effects. The review presents data on some clinical, genetic factors affecting the bioavailability and concentration of *TAC* in the blood. We also present data on the methodological aspects of *TAC* laboratory control.

Keywords: tacrolimus, P450 gene polymorphism, prescription protocols, drug monitoring, organ transplantation.

Combined immunosuppressive therapy is an important aspect of treating the patients after solid organ transplantation. Basic immunosuppressive drugs in recipients of solid organs include calcineurin inhibitors (tacrolimus, cyclosporin A), proliferative signal inhibitors (sirolimus, everolimus), corticosteroids, mycophenolic acid, etc.

The factors that play a role in the pharmacokinetic variability of tacrolimus (*TAC*) include patient characteristics (age or weight), polymorphism of genes encoding enzyme proteins involved in *TAC* metabolism [1]. The clear benefits of TAC must be balanced against its side effects. Besides, multiple drug interactions with inducers and inhibitors of cytochrome P450 3A (CYP3A) CYP3A4/A5 isoforms increase the risk of insufficient or excessive *TAC* effects.

The present review examines the clinical aspects of the pharmacodynamics of TAC, pharmacogenetic factors influencing the results of allotransplantation, laboratory monitoring of the drug concentration.

Tacrolimus known as FK-506 is a macrolide immunosuppressant isolated from *Streptomyces tsukubaensis*. The drug was obtained in 1984 by Japanese researchers [2]. *TAC* is a sustained release drug that inhibits calcineurin, a protein phosphatase necessary for the activation of T lymphocytes. Compared with cyclosporin A, *TAC* has a more pronounced antiproliferative effect and better tolerance. The current use of TAS exceeds that of cyclosporin A; its powerful immunosuppressive effect is 100 times stronger than that of cyclosporin A. Due to the fact that *TAC* is metabolized through the cytochrome P-450 system, its concentration in the blood can alter at the simultaneous administration of drugs using the same metabolic pathways [3, 4].

DRUG PHARMACODYNAMICS: ACTION MECHANISM

TAC has become one of the most commonly prescribed immunosuppressants after solid organs – heart, lungs, kidneys, and pancreas – transplants. TAS binds to FKBP-12, an immunophilin (FKBP12 – FK506 complex) responsible for signal transduction and forms a pentameric complex with Ca2+ calmodulin and calcineurin. The resulting formation inhibits the action of the nuclear factor activated T cells (NFAT). Expression of NFAT is required for the production of interleukin-2 (IL-2) to initiate the activation of T lymphocytes. *TAC* was found to not only inhibits the activation of T cells, but also reduces the production of IL-10, which prompts B cells to produce large amounts of antibodies. *TAC* can inhibit the release of inflammatory mediators and molecules from basophils and mast cells. The main mechanism of TAS action is to inhibit the redistribution of calcineurin in the slit diaphragm [5, 6].

DRUG PHARMACOKINETICS AND PHARMACOGENETICS

TAC is absorbed mainly in the small intestine, with food significantly affecting the relative bioavailability of the drug. Whereas the highest absorption occurs in fasting state, a diet high in fats and carbohydrates lowers the mean area under the curve (AUC) and maximum *TAC* concentrations in blood. *TAC* concentration in blood

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reaches its peak (Cmax) in about 1–3 hours; bioavailability varies in solid organ recipients from 4 to 93%, with 25% on average. *TAC* is primarily redistributed in erythrocytes. Concentrations in whole blood are 10–30 times higher than drug concentrations in plasma; therefore, the measurement of *TAC* in whole blood is most widely used in clinical practice. TAS is 99% bound to plasma proteins: albumin, α-1 acid glycoprotein (orosomucoid), lipoprotein, and globulins [7]. The pharmacokinetics of TAS are influenced by such factors as age, the patient ethnicity, the donor organ condition, comorbidities, medications, diet, and polymorphism of the drug metabolizing enzyme and carrier protein. *TAC* is almost completely metabolized by isoenzymes CYP3A4 and CYP3A5 in the liver and is a substrate of P-glycoprotein (P-gp) encoded by the multidrug resistance gene 1 (ABCB1) [8, 9]. Studies have shown that CYP3A5 is the predominant enzyme in *TAC* metabolism. Polymorphism of the CYP3A5 gene is the main cause of the toxic effect when taking *TAC*. Replacement of the A6986G nucleotide in the CYP3A5 gene (CYP3A5* 3 allele) leads to the lack of functional activity of CYP3A5 in liver tissue (CYP3A5 are not expressors). For patients with this phenotype, lower doses of *TAC* are required. Heterozygous or homozygous carriers of the wild-type CYP3A5* 1 allele, designated * 1 / * 1 and * 1 / * 3, produce high levels of CYP3A5 mRNA and protein (CYP3A5 expressors). With these phenotypes, higher doses of the drug may be required for patients taking *TAC* [10, 11].

IMMUNOSUPPRESSION IN CHILDREN

At immunosuppressive therapy in children, it is necessary to consider the features of children's immunity. The difference between the immune system of young children is the immaturity of T and B lymphocytes. Immature B cells are featured with producing only class M immunoglobulins. Maturation of B cells goes on during the first year of life and is reflected in the sequential appearance of different classes of immunoglobulins in the blood serum. IgA synthesis, especially its secretory form, is completely absent in newborns and appears after the 3rd month of life, which gives reason to speak about the insufficiency of the local immunity system in the first years of life. The suppressor function of the immune system of infants in the first year of life to the mother's lymphocytes is physiological and is aimed at preventing severe immunocomplex pathology which is possible upon contact with a large number of antigens [12]. In children, the half-life of *TAC* is two times shorter than in adults, the drug clearance rate is 2–4 times higher, and the volume of distribution is 1.8 times higher in the early post-transplant period [13]. When *TAC* is administered orally, some children require a longer period for drug accumulation, while others, on the contrary, quickly achieve the required therapeutic level. It has also been shown in children that the CYP3A5 polymorphism has a significant effect on the pharmacokinetic variability of *TAC*. Children with the CYP3A5* 1 allele have higher *TAC* dose requirements than CYP3A5 non-expressors. For children and adolescents with at least one CYP3A5* 1 allele, an increase of 1.5–2 times in dose is similar to the recommendation for adults. Although CYP3A5 may explain up to 45% of *TAC* pharmacokinetic variability between individuals, other factors can also influence *TAC*: differences in gastric emptying rate or *TAC* inability to dissolve in gastric contents. The effect of immunosuppressants on the growth and development of children, especially on the course of infectious processes and higher rates of post-transplant lymphoproliferative morbidity should be considered [14, 15].

TOOLS AND SCHEMES FOR IMMUNOSUPPRESSION SELECTION

The problem of selecting an immunosuppression regimen after organ transplantation is relevant due to the fact that recipients, on the one hand, when prescribing high doses of immunosuppressants, have a high risk of developing infectious complications, malignant neoplasms, and on the other hand, when prescribing minimal doses of immunosuppressants, transplant rejection and dysfunction may develop.

Initial immunosuppression is selected empirically based on the body weight of the recipient and the scheme of twice a day administration; further, biochemical parameters and the level of the drug in blood are considered. *TAC* can be administered in a variety of forms and schemes: intravenously, per os, twice daily with immediate release, once daily with modified release, and dose requirements change over time. This makes it necessary to develop various programs for selecting the drug dose based on population models of pharmacokinetics.

There are a number of electronic resources for routine selection and dose adjustment of various drugs including *TAC*, considering the therapeutic value prescribed by the attending physician [16]. The ISBA website (www.pharmaco.chu-limoges.fr) allows for individual dose adjustment of immunosuppressants. The user fills out a form in which a number of parameters are indicated, the type of transplanted organ, time between the administration of the drug and the measurement of the concentration, concomitant medications, etc. The request is confirmed within 24 hours by a qualified pharmacologist providing individual recommendations to achieve the therapeutic goals.

DoseMe (www.doseme.com.au) is another available tool suitable for dose adjustment of TAS and other drugs using previously published population pharmacokinetic models [17]. It is available as a website with a user interface. The program handles a variety of immunosuppression regimens: twice daily or once daily, based on the population model by Woillard et al. [18]. Other resources (MWPharm and BestDose) are computer software, all actions are performed online, and the user only has to

provide input data, which is automatically checked to exclude erroneous values and interpret the report.

Some medical centers are developing their own personalized treatment regimens based on immunosuppressive drugs for the patients with solid organ transplantation.

LABORATORY MONITORING IMMUNOSUPPRESSANTS

The severity of potential adverse events necessitates regular monitoring of the scheme of administering such basic immunosuppressive drugs as *TAC*, cyclosporin A, everolimus, etc., and their blood concentration in recipients of these drugs. Drugs are monitored in many laboratories using one of the methods, immunochemical analysis, or liquid chromatography with tandem mass spectrometry (LC-MS/MS) [19, 20].

As with many drugs, *TAC* levels tend to vary greatly among patients, depending on many factors, so there are no established reference values for its concentration. Test results should only be interpreted by a physician and used in conjunction with other diagnostics. In some cases, *TAC* can be used in combination with other immunosuppressants to reduce the effects of harmful side effects. In some cases, the patient needs to be kept at low *TAC* concentrations (3–7 ng/ml); for this, laboratories must use techniques with low sensitivity limits, from 1 ng/ml [21].

The study by the International Association Of Therapeutic Drug Monitoring And Clinical Toxicology (IATDMCT) identified LC-MS/MS as the "gold" drug monitoring technique due to its high specificity in 53% of the laboratories surveyed, 76 in 14 countries. This method allows the optimal separation of molecules into fragments [22].

Mass spectrometry can also be applied to analyze dried blood samples. Its advantage is that the samples are collected from the patients at home and then sent to the laboratory for measurement, thus reducing transport costs and saving time for the patient (the technique has not been registered in Russia). However, high hematocrit has been shown to affect paper permeability. Patient samples with increased cell volumes have lower paper permeability, creating a smaller stain affecting accurate results [23, 24].

TAC quantitative assessment at the immunochemical analysis is one of the advantages of the technique. Similar to LC-MS/MS, sample measurement in an immunoassay is carried out after a pretreatment step, an example is sample preparation for analysis with ARCHITECT i2000SR device (Abbott Diagnostics) by chemiluminescence which uses methanol/zinc sulfate to precipitate protein and extract *TAC* from a whole blood sample with ethylenediaminetetraacetic acid (EDTA). To assess *TAC* concentration in the blood, COBAS (Roche) and Dimension (Siemens Healthcare Diagnostics) analyzers can be used which imply the techniques of enzyme immunoassay and antibody-conjugated magnetic immunoassay.

The main disadvantage observed in many non-chromatographic systems is the potential cross-reactivity between the parent drug and its metabolites which can lead to falsely elevated blood drug concentrations [19].

Advances in immunochemical assay include automatic sample pretreatment, improved reagent stability to reduce potential matrix effects, and new anti-*TAC* antibodies that provide greater sensitivity and proximity to target concentration. Immunoassay is used in many laboratories because of its ease of use and reduced costs associated with services, the manufacturer often provides training, support, and service for these systems. LC-MS/MS testing requires high technical skills and extensive training. This technique also presumes a high initial cost and full validation to use [25].

ISSUES OF DRUG INTERACTIONS

The lifelong use of immunosuppressive drugs, on the one hand, improves the survival of recipients, and on the other hand, it leads to the problem of toxic side effects on the kidneys, heart and other organs against the background of long-term drugs administration. In patients with a low of risk, the dose of immunosuppressive drugs may be reduced for 1–2 years. Reducing the effect of nephrotoxicity usually means reducing the dose of calcineurin inhibitors and corticosteroids.

The concomitant diseases in the recipient (arterial hypertension, infectious complications, renal failure, etc.) is accompanied by the appointment of additional medications, which increases the risk of unwanted interactions. Additional prescription of drugs should be performed considering their potential effect (increase or decrease) on *TAC* concentration [3, 26].

CONCLUSION

The use of inhibitors of calcineurin, cyclosporin A and tacrolimus improved graft and patient survival rates, significantly reducing the incidence of acute and chronic rejection. However, long-term use of these drugs leads to the development of nephrotoxicity, metabolic and cosmetic side effects, as well as other possible complications (systemic arterial hypertension, neurotoxicity, an increased risk of developing infectious complications, the occurrence of post-transplant lymphoproliferative disorders).

However, the issues of developing approaches to the individualization of immunosuppressive therapy through studies of the pharmacokinetics of tacrolimus, including genetic aspects, as well as issues of drug interactions in recipients with comorbid pathology, remain relevant.

TAC laboratory monitoring is an important part of the post-transplant management of recipients and can be performed by two different methods, LC-MS/MS or immunochemical analysis. To ensure accurate and accu-

rate results, the selected blood TAC monitoring platform must be certified, standardized, and well supported.

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