

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Metabolic Drug Interactions with Immunosuppressants

Katalin Monostory

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.74524>

Abstract

Organ transplantation has become a routine clinical practice for patients with end-stage disease of liver, kidney, heart, or lung. Although improved immunosuppressant therapy substantially contributes to the success of transplantation, clinicians continue to face challenges because of wide interindividual variations in blood concentrations resulting in subtherapeutic or supratherapeutic levels. Many undesired side-effects or therapeutic failure of immunosuppressants as a consequence are the results of differences or changes in drug metabolism. Considering genetic and nongenetic factors, such as co-medication, can refine the immunosuppressant therapy, facilitating personalized treatments to individual recipients. This review provides an up-to-date summary of functional polymorphisms of enzymes involved in the metabolism of immunosuppressants with low molecular weight and of the clinical significance of metabolic drug interactions between immunosuppressive agents and other drugs in therapeutic regimens of transplant recipients.

Keywords: drug metabolism, genetic polymorphism, phenoconversion, calcineurin inhibitors, mTOR inhibitors, corticosteroids, inosine monophosphate dehydrogenase inhibitors

1. Introduction

Many undesired side-effects or therapeutic failures of drugs are the results of differences or changes in drug metabolism. A patient's drug metabolizing capacity, highly influenced by genetic variations or alterations in the expression and activities of drug-metabolizing enzymes, can substantially modify the pharmacokinetics of a drug and eventually its efficacy or toxicity [1]. Even if the routine clinical practice applies blood concentration guided dosing,

the interindividual variability in drug metabolism calls for personalized medication primarily for drugs with narrow therapeutic index [2, 3]. The identification of genetic and nongenetic factors that can potentially affect the pharmacokinetics of a particular drug is a prerequisite of tailored pharmacotherapy [4, 5].

2. Genetic and nongenetic variations of drug-metabolizing cytochrome P450 (CYP) enzymes

CYP enzymes are the key players in the metabolism of most drugs; therefore, interindividual and intraindividual variations in CYP activities are of significant importance in clinical practice. The pharmacokinetic variability can divide the population into poor, intermediate, extensive, and ultra-rapid metabolizer phenotypes. The loss-of-function mutations in CYP genes result in permanent poor metabolism, whereas nongenetic (internal or environmental) factors can substantially modify the expression and activities of CYP enzymes, evoking transient poor or extensive/ultra-rapid metabolism [6, 7]. The clinical relevance for many CYP genetic variants, regarding drug efficacy, adverse drug reactions, or dose requirement, has been clearly evidenced [6–9]; however, the heritable genetic polymorphisms are not the only determinant factors in interindividual differences in drug metabolism. CYP genotype determines the potential for the expression of functional or nonfunctional enzymes; and nongenetic host factors (age, sex, and disease states) and environmental factors (nutrition, medication, smoking, and alcohol consumption) can alter the expression and activities of CYP enzymes [10]. Homozygous wild genotype, predicted to be translated to functional CYP enzyme, can be transiently switched into poor or extensive metabolizer phenotype, due to phenoconversion [1, 11]. Consequently, both the CYP genotype and the current CYP expression or activity should be considered for the estimation of a patient's drug-metabolizing capacity.

The prevalence of loss-of-function or gain-of-function alleles is generally 1–10%; however, the distribution of the common CYP variants varies among different ethnic populations. CYP3A enzymes, responsible for the metabolism of approximately 40% of the drugs on the market, including many immunosuppressant agents, display great genetic and nongenetic variations. For CYP3A5, substantial interethnic differences in allelic variants have been demonstrated. The prevalence of CYP3A5*3 allele (6986A > G), resulting in splicing defect and nonfunctional CYP3A5 protein, is 88–97% in white (Caucasian), 66% in Asian, and 12–35% in African populations; consequently, a higher average proportion of functional CYP3A5 in the total hepatic CYP3A pool is expected in subjects of black origin [7, 12]. On the other hand, the enormous, even more than 100-fold interindividual variability in the expression and activity of CYP3A4 is attributed to nongenetic factors rather than genetic polymorphisms [13]. CYP3A4*1B allele, which has a frequency of 3–5% in white populations, but a much higher frequency in African population (50–82%) has been reported to result in increased transcription; however, the clinical significance of CYP3A4*1B to CYP3A4 function seems to be doubtful [14, 15]. CYP3A4*22 allele with the prevalence of 2.5–8% in white and of 4% in Asian populations displays low hepatic CYP3A4 expression and results in decreased CYP3A4 activity [16]. Although the association between CYP3A4 genotype and pharmacokinetic behavior of CYP3A-substrates has been extensively studied, no clear phenotype-genotype relationship has been described for CYP3A4.

Beside the genetic polymorphisms, one of the major sources of interindividual or intraindividual variability in drug metabolism is concomitant medication and co-morbidities, evoking phenoconversion, notably CYP induction and enzyme inhibition [17]. CYP induction leads to an increase in the expression and activity of CYP enzymes and contributes to the increased elimination of drugs metabolized by the particular enzyme. Several pathways involving the activation of various nuclear receptors (PXR pregnane X receptor, CAR constitutive androstane receptor, and glucocorticoid receptor) have been reported to enhance the transcription of CYP3A genes and to contribute to the complex regulation of CYP3A enzymes by drugs such as rifampicin, phenobarbital, carbamazepine, and synthetic or natural steroids [18–21]. Reduced drug concentration as a consequence of CYP3A induction leads to the lack of the pharmacological effect and drug failure. Phenoconversion converting genotypic extensive metabolism into phenotypic poor metabolism of drugs may occur during inflammation (sterile or infection-induced inflammation). Elevated release of proinflammatory cytokines (IL-6, IL-1 β , TNF- α) has been associated with downregulation of several drug-metabolizing CYPs, including CYP3A enzymes. The mechanism of downregulation is the repression of PXR and CAR that are involved in transcriptional regulation of CYP3A expression [22–26]. As a consequence, transient poor metabolizer phenotype is developed, significantly increasing the risk of adverse drug reactions and impacting the clinical outcome [1, 27]. Likewise, co-medication can also give rise to poor metabolism. Several drugs or food components (e.g., bergamottin) are known to inhibit the function of drug-metabolizing CYPs; therefore, the concomitant treatment with a CYP inhibitor is expected to increase the exposure of those pharmaceuticals that are metabolized by the particular enzyme. As a consequence of CYP inhibition, the risk of increased exposure and drug-induced adverse reactions can be anticipated, primarily for drugs with narrow therapeutic index, such as tacrolimus and ciclosporin.

By recognizing individual differences in drug metabolism, personalized drug therapy adjusted to the patient's drug-metabolizing capacity can help to avoid the potential side effects of drugs. The graft and recipient survival are highly influenced by drug-metabolizing capacity of the liver, and it is essential to predict potential drug-drug interactions and to tailor medication at both early and late postoperative periods.

3. Metabolism of immunosuppressants

In recent decades, transplantation (liver, kidney, heart, and lung) has become a routine procedure for patients with end stage disease. Advances in surgical techniques and postoperative therapy have led to increasing numbers of transplantation and extended survival among these patients. The final outcome of transplantation and the long-term graft function have been improved mainly due to the development of potent and specific immunosuppressive drugs. Immunosuppressants efficiently decrease the risk of rejection, blocking the recipient's immune system and protecting the transplanted organ. Because of the narrow therapeutic indexes and increased risk of adverse drug reactions, it is essential to apply personalized immunosuppressive therapy adjusted to patient's drug-metabolizing capacity.

Immunosuppressants are generally classified according to their molecular mode of action; however, in terms of metabolic drug interactions, two main categories must be distinguished

| Immunosuppressant | Pharmacology | Adverse effects | Enzymes responsible for the metabolism |
|--|---|---|---|
| <i>Calcineurin inhibitors:</i> | | | |
| Ciclosporin | Selective inhibition of T-cell dependent immune response: Calcineurin inhibition, Inhibition of cytokine production | Nephrotoxicity, hepatotoxicity, Hyperlipidaemia, hypertension, Tremor, hyperkalaemia, hypomagnaesemia, Hypertrichosis, gingiva hyperplasia | CYP3A4/5 |
| Tacrolimus | Selective inhibition of T-cell dependent immune response: Calcineurin inhibition, Inhibition of cytokine production | Nephrotoxicity, hypertension, diabetes, cholestasis, diarrhea, Tremor, hyperkalaemia, hypomagnaesemia | CYP3A4/5 |
| <i>mTOR inhibitors:</i> | | | |
| Sirolimus | Inhibition of B- and T-cell proliferation | Thrombocytopenia, anaemia, leukopenia, lymphocele, pneumonitis Hyperlipidaemia, Stomatitis aphthosa, wound-healing complications | CYP3A4/5 |
| Everolimus | Inhibition of B- and T-cell proliferation | Thrombocytopenia, anaemia, leukopenia, lymphocele, pneumonia Hyperlipidaemia, hypertonia, wound-healing complications | CYP3A4/5 |
| <i>Purine analogues:</i> | | | |
| Azathioprin | Inhibition of purine metabolism | Bone marrow suppression, leukopenia, anaemia, thrombocytopenia, myeloid dysplasia, Cholestasis, hepatotoxicity | Thiopurine S-methyl-transferase, Xantine oxidase |
| <i>Inosine monophosphate dehydrogenase inhibitors:</i> | | | |
| Mycofenolate mofetil Mycofenolate | Selective inhibition of inosine monophosphate dehydrogenase, Inhibition of B- and T-cell proliferation | Vomiting, diarrhea, abdominal pain muscle weakness, Anaemia, leucopenia | UDP-glucuronyl transferase, CYP3A4/5 |
| <i>Corticosteroids:</i> | | | |
| Prednisone Methyl-prednisolone | Inhibition of T-cell migration and production of T-cell lymphokines | Adrenal cortex suppression Hypercholesterolemia, diabetes, hypertension, osteoporosis, osteonecrosis, cataracta, skin atrophy | CYP3A4/5 |

Table 1. Immunosuppressants with low molecular weight.

according to their molecular weights (agents with low or high molecular weights). High-molecular-weight agents, such as polyclonal and monoclonal antibodies (e.g., thymoglobulin, basiliximab, belatacept), that are not substrates for drug-metabolizing enzymes, are metabolized in common protein degradation pathways (intracellular catabolism by endosomal-lysosomal system) [28]; therefore, they are not subjects of metabolic drug interactions and not subjects of this review. In the metabolism of immunosuppressants with low molecular weight, drug-metabolizing CYP enzymes are involved which may entail metabolic drug interactions (**Table 1**).

3.1. Calcineurin inhibitors

For solid organ transplant recipients, the mainstay of the immunosuppressive regimens is calcineurin inhibitor (CNI) therapy with ciclosporin or tacrolimus which selectively blocks several signaling processes, resulting in the inhibition of T-cell activation and proliferation (**Figure 1**) [29, 30]. These drugs effectively treat allograft rejection; however, they display large interindividual variability in their pharmacokinetics, requiring monitoring of blood concentrations for optimal safety and therapeutic efficacy.

Ciclosporin A is an 11-amino acid cyclopeptide that blocks the production of IL-2 by inhibition of calcineurin and, as a consequence, the activation of T-cells (**Figure 1**) [31]. Ciclosporin undergoes extensive metabolism by CYP3A enzymes, producing more than 30 metabolites. The major metabolic pathways are *N*-demethylation to 4-*N*-demethyl ciclosporin, hydroxylation at several positions (1-, 6-, 9-monohydroxy and 1,9- or 6,9-dihydroxy-metabolites), and oxidation to carboxylic acid [32]. Some of the metabolites (e.g., 1,9-dihydroxy-ciclosporin,

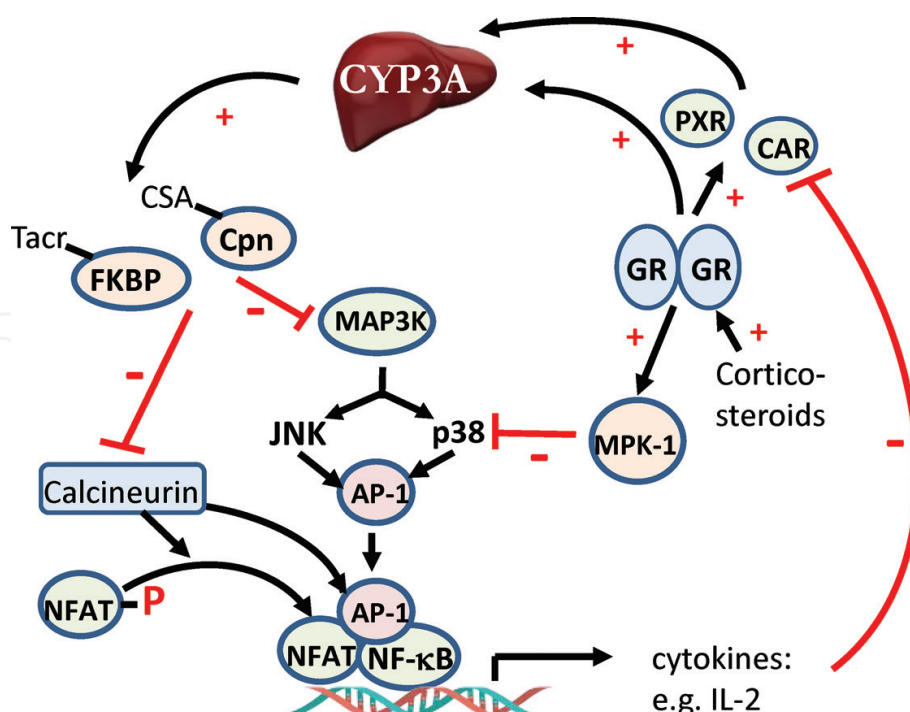


Figure 1. Molecular action of calcineurin inhibitors and corticosteroids. AP-1 activator protein 1, CAR constitutive androstane receptor, CSA ciclosporin, FKBP tacrolimus binding protein, GR glucocorticoid receptor, IL-2 interleukin 2, JNK c-Jun N-terminal kinase, MAP3K mitogen-activated protein 3 kinase, MPK-1 mitogen-activated protein kinase 1, NFAT nuclear factor of activated T-cells, PXR pregnane X receptor, Tacr tacrolimus.

1c9-dihydroxy-ciclosporin, 1-carboxy-ciclosporin) are toxic contributing to the nephrotoxic and hepatotoxic properties of the parent compound [33, 34]. Consequently, high CYP3A activity increases the rate of ciclosporin metabolism and decreases the immunosuppressive effect, which requires dose modification [35]. However, high CYP3A activity also increases the toxic metabolite formation and the risk of nephrotoxicity and hepatotoxicity. Therefore, immunosuppressive strategy must consider the blood concentrations of both ciclosporin and the toxic metabolites, especially if they are accompanied with symptoms indicating nephrotoxicity or hepatotoxicity.

Immunosuppressive properties of tacrolimus are similar to ciclosporin; however, for the same pharmacological effect, significantly lower blood concentration of tacrolimus is required than that of ciclosporin. Tacrolimus, the 23-membered macrocyclic lactone, is converted by demethylation, hydroxylation, and ring rearrangement to at least 15 metabolites, and only a minor proportion of tacrolimus dose is eliminated as unchanged parent drug [36]. Metabolism of tacrolimus leads to the inactivation of the molecule, except for the major 13-*O*-demethyl and the minor 31-*O*-demethyl metabolites. The 13-*O*-demethyl-tacrolimus possesses some immunosuppressive effect; however, it is about one tenth as active as tacrolimus, whereas the 31-*O*-demethyl metabolite displays an immunosuppressive activity comparable to tacrolimus [37, 38]. On the other hand, high blood concentration of 15-*O*-demethyl-tacrolimus metabolite has been reported to be associated with nephrotoxicity and myelotoxicity and with higher incidence of infections [39]. Similarly to ciclosporin, tacrolimus is metabolized by CYP3A enzymes, anticipating great interindividual and intraindividual differences in pharmacokinetics of tacrolimus: (1) CYP3A activity of enterocytes contributes to the first-pass metabolism of tacrolimus; (2) substantial interindividual differences in hepatic CYP3A activity result in great variability in the rate of tacrolimus metabolism, which requires continuous drug monitoring and dose modification primarily in the early postoperative period; (3) concomitant treatment with CYP3A inhibitors is the potential source of metabolic drug interactions; (4) genetic polymorphisms of CYP3A5 also contribute to the high interindividual variability. Since the relative contribution of CYP3A5 to tacrolimus biotransformation is significantly higher than that of CYP3A4 [40], the recipients carrying wild type *CYP3A5*1* allele or transplanted with liver grafts carrying *CYP3A5*1* are able to metabolize tacrolimus more rapidly than CYP3A5 nonexpressers [35, 41].

3.2. mTOR (mammalian target of rapamycin) inhibitors

The mTOR inhibitors prevent cell proliferation by blocking cell cycle progression from the G1-phase to the S-phase. The immunosuppressive activity is mediated *via* blocking mTOR protein kinases, resulting in inhibition of growth factor-mediated T-cell proliferation in response to IL-2 trigger [42]. Sirolimus is a 31-membered macrolide, whereas everolimus is a sirolimus derivative having a 2-hydroxyethyl chain substitution at position 40. Although the chemical structures of sirolimus and everolimus are similar to that of tacrolimus, the mechanism of action of mTOR inhibitors is distinct from calcineurin inhibitors, which allows the application of combination regimens. Additionally, the main advantages of mTOR inhibitors are their nonnephrotoxic properties; therefore, mTOR inhibitors in combination with reduced dose calcineurin inhibitors can augment the calcineurin inhibitor-induced nephrotoxicity [43–45].

The structural similarities can explain some common metabolic pathways of mTOR inhibitors and tacrolimus, such as *O*-dealkylation and hydroxylation at several positions [42]. Sirolimus is primarily metabolized by CYP3A enzymes and by CYP2C8 at lower extent, producing hydroxylated and *O*-demethylated metabolites (e.g., 12-hydroxy-, 16-*O*-demethyl-, 39-*O*-demethyl-, 27–39-*O*-didemethyl- and dihydroxy-sirolimus as major metabolites) [46, 47]. The metabolism of sirolimus leads to inactivation, despite the fact that some metabolites display some pharmacological activity less than one tenth of the parent drug. Everolimus is also metabolized by CYP3A and CYP2C8 enzymes; however, the elimination rate of everolimus is more rapid than sirolimus (with 30 h *vs.* 62 h elimination half-lives, respectively). Everolimus is *O*-demethylated and hydroxylated at several positions (forming both mono- and dihydroxy-metabolites); furthermore, a ring-opened metabolite is also formed from everolimus [46]. Everolimus-induced adverse effects are associated with the exposure rather to the parent compound than to its metabolites.

3.3. Antimetabolite purine analogues

One of the oldest agents with immunosuppressive activity introduced for kidney transplant recipients was the purine analogue 6-mercaptopurine, which acts by inhibiting purine nucleotide synthesis and, as a consequence, cell proliferation. The prodrug of 6-mercaptopurine, azathioprine with more favorable side-effect profile was later introduced to prevent rejection. Azathioprine is converted to 6-mercaptopurine by nonenzymatic cleavage of the thioether in enterocytes and hepatocytes or in erythrocytes. The major active metabolites, 6-thioguanine nucleotides, are formed *via* 6-thioinosine monophosphate in natural purine synthetic pathways. Inhibition of cell proliferation is mediated by incorporation of the thiopurine nucleotide analogues into DNA (and RNA), causing DNA damage [48]. 6-Mercaptopurine, independently from either direct administration or production from azathioprine, undergoes metabolic inactivation by xanthine oxidase and thiopurine S-methyl transferase and is excreted in the urine, leaving less parent compound available to form thiopurine nucleotides [49]. Due to genetic polymorphism, the thiopurine S-methyl transferase activity is highly variable in patients; namely, those subjects who carry one or two nonfunctional thiopurine S-methyl transferase alleles are unable to tolerate normal doses of azathioprine and can experience serious myelosuppression [50]. Therefore, genotyping assay is recommended before starting azathioprine therapy to identify high-risk patients, and dosage reduction or alternative therapy is recommended for these patients.

3.4. Inosine monophosphate dehydrogenase inhibitors

Mycophenolic acid is a selective inhibitor of inosine monophosphate dehydrogenase, which is responsible for *de novo* biosynthesis of guanosine monophosphate, one of the building blocks of DNA. Depletion of the guanosine pool in the cell arrests the lymphotic cell proliferation and suppresses the subsequent immune response triggered by allogenic transplanted organ [51]. In several rapidly dividing cells (e.g. enterocytes), an alternative salvage pathway exists for purine synthesis in addition to *de novo* synthetic pathway; however, lymphocytes seem to be dependent on the *de novo* pathway. Consequently, mycophenolic acid is able to selectively block proliferation of T- and B-cells. Mycophenolic acid is available as enteric-coated mycophenolate sodium and as mycophenolate mofetil ester prodrug that is extensively hydrolyzed to the active metabolite mycophenolic acid by carboxylesterases.

Mycophenolic acid is primarily metabolized by UDP-glucuronyl transferases (UGT1A7/8/9, UGT2B7), forming the major 7-*O*-mycophenolic glucuronide that is pharmacologically inactive and to the minor acil-glucuronide that has pharmacological activity comparable to the mycophenolic acid [52, 53]. The major proportion of the glucuronide conjugates is excreted in urine, whereas a smaller proportion that is eliminated *via* bile is metabolized by bacteria in the gut, and the deconjugated mycophenolic acid can be reabsorbed (enterohepatic circulation) [54]. Furthermore, in patients' blood and urine, a minor demethylated metabolite (6-*O*-demethyl-mycophenolic acid) was also detected that was proved to be produced by CYP3A enzymes.

3.5. Corticosteroids

At the beginning of transplantation history, glucocorticosteroids were the primary immunosuppressive agents in the rejection prophylaxis strategy, and nowadays, they are still the first-line agents for treatment of graft rejection. The high-dose glucocorticoids given in peritransplantation are tapered to low doses in the maintenance phase, aiming the steroid-free immunosuppression regimens because of serious adverse effects of glucocorticoids developing in long-term therapy. Acute rejection is generally treated with methylprednisolone, whereas the maintenance therapy applies either methylprednisolone or prednisone. Corticosteroids activate the cytosolic glucocorticoid receptor and modulate several cellular functions, including transcription of genes involved in proliferative and inflammatory processes. The activated receptor inhibits the transcription of NF- κ B and activator protein 1 dependent genes, including proinflammatory cytokines (**Figure 1**). This process leads to the depletion of T-cells and macrophage dysfunction [55].

Regioselective and stereospecific hydroxylation of corticosteroids at several positions (at carbon 2, 6, 7, 15, 16, and 21) are catalyzed by CYP3A enzymes. Additionally, dual effect of corticosteroids on CYP3A enzymes has been demonstrated: (1) corticosteroids can competitively inhibit the function of CYP3A [56], and (2) they can induce CYP3A transcription. Activated glucocorticoid receptor upregulates the expression of nuclear receptors (PXR and CAR) that are involved in transcriptional regulation of CYP3A genes. Moreover, the proximal promoter region of CYP3A4 gene contains glucocorticoid responsive element, which directly binds activated glucocorticoid receptor [18, 57]. As a consequence of increased expression and activity of CYP3A enzymes, metabolic drug interactions can be expected upon concomitant treatment with drugs that require CYP3A activity for their metabolism.

3.6. Novel investigational immunosuppressant agents

Although calcineurin inhibitor-based immunosuppression efficiently prevents rejection, adverse reactions of ciclosporin and tacrolimus, primarily nephrotoxicity, prompt the discovery of novel agents with immunosuppressive activity [58]. Two investigational agents with low molecular weight should be mentioned: voclosporin and sotrastaurin. Voclosporin, a next-generation calcineurin inhibitor, is an analogue of ciclosporin with a single carbon extension added to the amino acid-1 of ciclosporin. Voclosporin displays higher binding affinity to cyclophilin A than ciclosporin leading to more potent inhibition of calcineurin [59]. Furthermore, it has a favorable safety property that it appears to be less toxic than currently available calcineurin

inhibitors. Similarly to ciclosporin, voclosporin is a substrate for CYP3A enzymes, anticipating pharmacokinetic/metabolic drug interactions with those agents that interact with ciclosporin as well [60]. However, voclosporin is no longer pursued in transplantation. Sotrastaurin is protein kinase C inhibitor that effectively inhibits IL-2 production with the mechanism different from calcineurin or mTOR inhibition. Although sotrastaurin displayed some potential in preventing allograft rejection in animal studies, high efficacy and safety failure rate were observed in clinical trials involving kidney and liver transplant patients [61, 62]. Therefore, further development of sotrastaurin in transplantation has been halted.

4. Significant metabolic drug interactions with immunosuppressants

4.1. Combined immunosuppressive therapy

Transplant recipients' immunosuppressive therapy is often a multidrug therapy, primarily in the early postoperative period, which constitutes a challenge for clinicians to consider the complexity of drug interactions. Due to the fact that the metabolism of immunosuppressants with low molecular weight is catalyzed by the same enzymes (CYP3A4 and CYP3A5), the blood concentrations, elimination half-lives, and consequently, the efficacy or toxicity of certain immunosuppressant agents are expected to be modified during concomitant treatment. Therefore, during multidrug therapy or during withdrawal of any of the immunosuppressive drugs, special attention is required for optimal dosing for therapeutic concentrations. Each modification in immunosuppressive regimens can lead to changes in blood concentration of a drug (**Table 2**).

Calcineurin inhibitors are often applied in combination with mTOR inhibitors. Since both mTOR inhibitors and calcineurin inhibitors are substrates of CYP3A enzymes and can inhibit CYP3A activities, reduction of calcineurin inhibitor doses is recommended. Standard doses of ciclosporin were observed to decrease the clearance of sirolimus or everolimus more substantially than the doses of tacrolimus [45]. The major drawback of calcineurin inhibitor therapy is the risk of nephrotoxicity which appears to be dose dependent. The combination of low calcineurin inhibitor doses with mTOR inhibitors was found to be beneficial regarding retaining low rejection rates and lowering the risk of nephrotoxicity [44, 63]. To avoid renal dysfunction, the complete substitution of calcineurin inhibitors for mTOR inhibitors was attempted; however, the substitution showed an increase in graft failure in patients treated with merely mTOR inhibitors [64].

Corticosteroids have been demonstrated to induce the expression of the efflux pump transporter ABCB1 (P-glycoprotein) playing a main role in intestinal drug absorption and of CYP3A enzymes responsible for the metabolism of the majority of drugs [18, 65]. Therefore, the concomitant treatment of calcineurin inhibitors or mTOR inhibitors with corticosteroids can be expected to decrease the blood concentrations of tacrolimus/ciclosporin or of sirolimus/everolimus. Although the evidence for clinically significant interactions between corticosteroids and ciclosporin or mTOR inhibitors is limited, clear clinical effect of corticosteroids on tacrolimus exposure has been demonstrated [66, 67]. This also implies that dose reduction or cessation of corticosteroids leads to an increase in blood concentrations of tacrolimus, requiring dose

| Immunosuppressant | Drug interactions | Consequences |
|---------------------------|---|---|
| Ciclosporin Tacrolimus | sirolimus, everolimus | Increased blood levels of ciclosporin and mTOR inhibitors; increased risk of nephrotoxicity |
| | prednisolone | Decreased blood levels due to enhanced metabolism of ciclosporin/tacrolimus; increased risk of rejection |
| | <i>Antifungals:</i> ketoconazole | Increased blood levels of ciclosporin/tacrolimus; replacement of ketoconazole to other azole derivatives |
| | fluconazole, voriconazole, itraconazole | Inhibition of CYP3A4; dose reduction of ciclosporin, tacrolimus is necessary |
| | <i>Antibiotics:</i> clarithromycin, erythromycin, azithromycin | Irreversible inhibition of CYP3A4; increased blood levels of ciclosporin/tacrolimus |
| | rifampicin | CYP3A4 induction; enhanced metabolism of ciclosporin, tacrolimus; increased risk of rejection |
| | <i>Antiviral agents:</i> ritonavir | Irreversible inhibition of CYP3A4; increased blood levels of ciclosporin/tacrolimus |
| | <i>Lipid-lowering agents:</i> fluvastatin, simvastatin, atorvastatin | Increased statin exposure by ciclosporin; increased risk of myopathy and rhabdomyolysis |
| | <i>Antihypertensive agents:</i> diltiazem, verapamil, amlodipine | Irreversible inhibition of CYP3A4, formation of metabolic intermediate complex; Increased blood levels of ciclosporin / tacrolimus |
| | nifedipine | Reversible, competitive inhibition CYP3A4 |
| | carvedilol | Inhibition of ABCB1 transporter; increase absorption of oral ciclosporin |
| | <i>Antidiabetic agents:</i> troglitazone, rosiglitazone | CYP3A4 induction; enhanced metabolism of ciclosporin/tacrolimus; increased risk of rejection |
| | <i>Psychopharmacocons:</i> carbamazepine, valproic acid | CYP3A4 induction; enhanced metabolism of ciclosporin/tacrolimus; increased risk of rejection |
| | fluvoxamine | Inhibition of CYP3A4; contraindicated |
| | <i>Herbs:</i> St John's wort | CYP3A4 induction; enhanced metabolism of ciclosporin/tacrolimus; increased risk of rejection |
| | grapefruit, pomelo | Irreversible inhibition of CYP3A4; increased blood levels of ciclosporin/tacrolimus |

| Immunosuppressant | Drug interactions | Consequences |
|---|---|---|
| Sirolimus Everolimus | ciclosporin | Increased blood levels of ciclosporin and mTOR inhibitors; increased risk of nephrotoxicity |
| | prednisolone | Decreased blood levels due to enhanced metabolism of sirolimus/everolimus, increased risk of rejection |
| | <i>Antifungals:</i> ketoconazole | Increased blood levels of mTOR inhibitors; replacement of ketoconazole to other azole derivatives |
| | fluconazole, voriconazole, itraconazole | Inhibition of CYP3A4; dose reduction of sirolimus, everolimus is necessary; voriconazole – sirolimus combination is contraindicated |
| | <i>Antibiotics:</i> clarithromycin, erythromycin, azithromycin | Irreversible inhibition of CYP3A4; increased blood levels of sirolimus/everolimus |
| | rifampicin | CYP3A4 induction; enhanced metabolism of sirolimus/everolimus; increased risk of rejection |
| | <i>Antiviral agents:</i> ritonavir | Irreversible inhibition of CYP3A4; increased blood levels of sirolimus/everolimus |
| | <i>Antihypertensive agents:</i> diltiazem, verapamil, amlodipine | Irreversible inhibition of CYP3A4, formation of metabolic intermediate complex; Increased blood levels of sirolimus/everolimus; verapamil-sirolimus combination is associated with increased blood levels of verapamil |
| | <i>Antidiabetic agents:</i> troglitazone, rosiglitazone | CYP3A4 induction; enhanced metabolism of sirolimus/everolimus; increased risk of rejection |
| | <i>Psychopharmaco</i> ns: carbamazepine, valproic acid | CYP3A4 induction; enhanced metabolism of sirolimus/everolimus; increased risk of rejection |
| | <i>Herbs:</i> St John's wort | CYP3A4 induction; enhanced metabolism of sirolimus/everolimus; increased risk of rejection |
| | grapefruit, pomelo | Irreversible inhibition of CYP3A4; increased blood levels of sirolimus/everolimus |
| 6-mercaptopurine Azathioprine Mycophenolate | allopurinol Ciclosporin | Inhibition of xantine oxidase; myelotoxicity Inhibition of enterohepatic circulation, decrease in blood levels of mycophenolic acid |

| Immunosuppressant | Drug interactions | Consequences |
|------------------------------------|--|---|
| Prednisolone Methylprednisolone | <i>Antiviral agents:</i> ganciclovir, valganciclovir | Mycophenolate-glucuronide inhibits renal tubular secretion of ganciclovir; increased blood levels of ganciclovir and increased risk of toxicity (nephrotoxicity, neutropenia, leukopenia) |
| | <i>Antifungals:</i> ketoconazole, fluconazole, voriconazole, itraconazole | Increased blood levels of corticosteroids Inhibition of CYP3A4 |
| | <i>Antibiotics:</i> rifampicin | CYP3A4 induction; enhanced metabolism of corticosteroids |
| | <i>Antiviral agents:</i> ritonavir | Irreversible inhibition of CYP3A4; increased blood levels of corticosteroids |
| | | |
| | | |

Table 2. Clinically relevant pharmacokinetic drug interactions with immunosuppressants.

adjustment [68]. Interestingly, CYP3A5 nonexpressers with *CYP3A5*3/*3* genotype are more susceptible to glucocorticoid induction than *CYP3A5*1* carriers [69]; thus, more pronounced increase in tacrolimus exposure can be expected in CYP3A5 nonexpressers after glucocorticoid withdrawal.

Clinically significant interaction between mycophenolic acid, the active metabolite of mycophenolate mofetil, and ciclosporin has been reported [70]. The mycophenolate-glucuronide metabolite eliminated into bile undergoes enterohepatic cycling because of intestinal bacterial metabolism and reabsorption of mycophenolic acid. The enterohepatic circulation, contributing to overall pharmacokinetics of mycophenolic acid by 37% in human, is inhibited by concomitant administration of ciclosporin but does not interfere with tacrolimus or sirolimus [71, 72]. In ciclosporin-mycophenolate combination therapy, the reduced blood concentration of mycophenolic acid is necessary to ameliorate by increasing dose of mycophenolate mofetil. Furthermore, special attention on optimal dosing is required during switching ciclosporin-mycophenolate to tacrolimus-mycophenolate therapy and *vice versa*.

4.2. Metabolic drug interactions between immunosuppressants and post-transplant medication

4.2.1. Treatment and prevention of infections

Environmental circumstances and immune deficiencies due to immunosuppression therapy make recipients susceptible for infections that are one of the leading complications after organ transplantation; therefore, prevention and management of infections is a major task

primarily in the early postoperative period. Since fungal infections are a threatening cause of morbidity and mortality, the antifungal prophylaxis is an important element of posttransplant medication. The antifungal azole-derivatives are potent (some of them are very strong) CYP3A inhibitors, predicting potential metabolic drug interactions with calcineurin inhibitors, mTOR inhibitors, or corticosteroids. The most potent CYP3A inhibitor is ketoconazole, able to increase blood concentrations (AUC) of ciclosporin (> 4-fold), tacrolimus (> 2-fold), sirolimus (11-fold), everolimus (15-fold), and methylprednisolone (> 2-fold) [73, 74]. Because of the substantial increase in blood concentrations of several immunosuppressants that can be avoided by drastic reduction of immunosuppressant doses and because of other adverse effects of ketoconazole, the concomitant medication is discouraged. Fluconazole, itraconazole, and voriconazole are alternative regimens for antifungal therapy or prophylaxis; however, all three drugs are azole derivatives and have the capability to inhibit CYP3A function, albeit at a lower extent than ketoconazole [75–77]. Although the continuous immunosuppressant monitoring is highly recommended and dose adjustment (reduction) is generally required, the antifungal treatment with fluconazole, itraconazole, or voriconazole can be safely applied except for voriconazole-sirolimus combination [78]. Because of an extreme (7-fold) increase of sirolimus blood concentrations as a consequence of concomitant use of voriconazole, this combination is contraindicated. Amphotericin B, the nonazole type antifungal agent, does not influence CYP activities; therefore, no metabolic drug interactions can be expected in concomitant treatment with immunosuppressants. However, the widespread use of amphotericin B is limited because of its toxicity profile, primarily because its nephrotoxic side-effect can contribute to the renal injury by ciclosporin or tacrolimus.

Organ transplant patients are at high risk for developing bacterial infections that occur in 20–40% of transplants. Potential sources of infection are from hospital and community exposures, as well as from endogenous flora of patients. Among the antibiotics used for treatment of infections, the macrolide erythromycin and clarithromycin have been reported to interact with immunosuppressive agents. These macrolides are CYP3A substrates and bind to CYP3A4 enzymes, leading to a complex formation that completely inactivates CYP3A4 enzyme [79–82]. The *in vitro* findings were confirmed by clinical observations that blood concentrations of ciclosporin/tacrolimus or sirolimus/everolimus increased as a consequences of concomitant treatment with erythromycin or clarithromycin [73, 83–86]. Page et al. [87] and Mori et al. [88] have reported some potential of azithromycin for drug interaction with ciclosporin and tacrolimus; however, *in vitro* experiments demonstrated that azithromycin poorly interfere with CYP3A4 [89]. When concomitant therapy with these macrolides is necessary, blood concentrations of calcineurin inhibitors or mTOR inhibitors should be carefully monitored, and the immunosuppressant doses should be adjusted. In contrast, the macrolide rifampicin is a potent CYP3A4 inducer and can activate PXR, resulting in a substantial increase in CYP3A4 expression [90]. The increased CYP3A4 activity consequently enhances the metabolism and elimination of calcineurin inhibitors, mTOR inhibitors, and corticosteroids [91–93]. However, blood concentration-guided dose-adjustment of immunosuppressants should be applied carefully because increased metabolism can evoke elevation of toxic metabolite formation (e.g., ciclosporin).

A significant cause of graft failure still remains viral infections, which are acquired as new infection or reactivation of latent viruses. After transplantation, cytomegalovirus (CMV) is the

most common viral infection in recipients, primarily in those CMV-seronegative patients who were transplanted with graft from CMV-seropositive donors, resulting in viral reactivation. For prophylaxis and treatment of CMV infection, aciclovir, ganciclovir, and valganciclovir (the prodrug of ganciclovir) are generally applied. None of these antiviral drugs influences the function of drug-metabolizing CYPs or UDP-glucuronyl transferases, and consequently, they do not modify the pharmacokinetic properties of immunosuppressants. Aciclovir and ganciclovir are eliminated primarily in the urine as unchanged compounds. Increased risk of nephrotoxicity and leukopenia has been reported in patients who were co-medicated with a drug that can reduce renal clearance of aciclovir or ganciclovir. During co-administration with mycophenolate or mycophenolate-mofetil, mycophenolate-glucuronide and aciclovir or ganciclovir can significantly compete for renal tubular secretion, resulting in an increase in aciclovir/ganciclovir and mycophenolate-glucuronide exposure, as well as the risk of nephrotoxicity or leukopenia [94–96]. Management of potent metabolic drug interactions between antiviral protease inhibitors and immunosuppressants is a major challenge because most of the protease inhibitors are clinically significant CYP3A4 inhibitors. Ritonavir-boosted therapies require substantial reduction of immunosuppressant doses (to 5–20% for ciclosporin; to 1–3.5% for tacrolimus) with continuous monitoring of blood concentrations [97–101].

4.2.2. *Treatment of dyslipidemia*

Dyslipidemia is often developed as an adverse impact of immunosuppressive therapy [102]. Ciclosporin, mTOR inhibitors, and prednisone are mainly implicated in lipid alterations. For treatment of hypercholesterolemia, the basic guidelines for dyslipidemia recommend diet and HMG-CoA reductase (hydroxymethyl-glutaryl-CoA reductase) inhibitor statins with special considerations for transplant patients. Although both ciclosporin and most statins (atorvastatin, fluvastatin, simvastatin, lovastatin) are primarily metabolized by CYP3A4 and metabolic drug interactions are likely occur, statins do not evoke increased ciclosporin exposure [103–106]. In contrast, ciclosporin induces significant elevation of statin blood concentrations which can be explained by the ten-fold higher molar concentrations of ciclosporin than statins. In combination with ciclosporin, the blood levels are increased in a statin-dependent manner, e.g., lovastatin is increased to a much greater extent than atorvastatin [104, 107]. Dose reduction of lipid-lowering agents is recommended to avoid myopathy or rhabdomyolysis. The blood concentrations of macrolide immunosuppressants (tacrolimus, sirolimus, and everolimus) are similar to that of statins [108, 109]; therefore, the lack of clinically relevant interactions between macrolides and statins is not unexpected.

4.2.3. *Antihypertensive agents*

Organ transplantation and immunosuppressive therapy (e.g., ciclosporin, prednisone) frequently trigger hypertension or worsen the preexisting disease in patients. While most of the antihypertensive agents (β -adrenoceptor blockers, α 1-adrenergic receptor antagonists, central α 2-adrenergic receptor agonists, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers) are not expected to influence the pharmacokinetics of immunosuppressants, medication with diltiazem, verapamil, or amlodipine requires special consideration and

frequent monitoring of immunosuppressant blood concentrations. The metabolism of all three Ca-channel blockers is primarily catalyzed by CYP3A4, anticipating potential drug interactions with immunosuppressants. Furthermore, significant inhibition of CYP3A4 by diltiazem, verapamil, and amlodipine has been demonstrated with an additional inhibitory property of metabolite intermediate complex formation that catalytically inactivates CYP3A4 and CYP3A5 enzymes [79, 80, 82, 110–114]. The inactivation of CYP3A enzymes by comedication with these antihypertensive drugs consequently leads to a permanent increase in blood concentrations of calcineurin inhibitors or mTOR inhibitors [115–122]. In transplant recipients comedicated with sirolimus and verapamil, an increase of blood concentrations of both sirolimus and verapamil was observed [123]. Furthermore, in patients carrying wild-type CYP3A5*1 allele, concomitant treatment with amlodipine significantly decreased tacrolimus clearance, and along with the changes in tacrolimus pharmacokinetics, an increase in amlodipine blood concentrations was also observed [124]. The metabolism of the Ca-channel blocker nifedipine is catalyzed almost exclusively by CYP3A enzymes, and competition for the active site of CYP3As may be expected if nifedipine and CYP3A substrate calcineurine inhibitors or mTOR inhibitors are concomitantly applied. In contrast, no evidence for pharmacokinetic drug interactions has been provided in transplant recipients treated with nifedipine and ciclosporin/tacrolimus or sirolimus/everolimus. Carvedilol is often used for treatment of hypertension in transplant patients, and pharmacokinetic drug interaction between carvedilol and ciclosporin has been observed that required 10–20% reduction of ciclosporin doses to maintain the blood concentrations within the therapeutic range [125, 126]. The major metabolic pathways of carvedilol are catalyzed by CYP2D6 and CYP1A2 rather than by CYP3A4 [127]; however, inhibition of CYP3A enzymes by carvedilol does not account for pharmacokinetic drug interaction with ciclosporin. Carvedilol has been demonstrated to block the function of the ABCB1 transporter protein (ATP-binding cassette B1; previously called as Pgp) [128]. In the intestinal wall, ABCB1 transporter pumps pharmacons or other xenobiotics passed into the enterocytes back into the gut lumen. The inhibition of ABCB1-mediated transcellular transport in the intestine by carvedilol is responsible for the increased absorption of ciclosporin. Under careful monitoring of ciclosporin blood concentration, the ABCB1 inhibition by carvedilol can be beneficial in ciclosporin-sparing therapy for transplant patients. Since the absorption of tacrolimus and mTOR inhibitors is also mediated by ABCB1, similar pharmacokinetic drug interactions between these immunosuppressants and carvedilol are presumably developed as with ciclosporin.

4.2.4. Antihyperglycemic therapy

Hyperglycemia developing posttransplant diabetes mellitus is generally medication related. Corticosteroids can evoke reduction of glucose tolerance, whereas ciclosporin and tacrolimus directly block insulin-release by islet cells. The metabolism of the sulfonylurea type antidiabetic agents (e.g., tolbutamide, glipizide, glibenclamide, and glimepiride) is mediated by CYP2C9; therefore, metabolic drug interactions with immunosuppressants are not expected in patients treated with any of these oral hypoglycemic drugs. Although the thiazolidinedione type troglitazone and rosiglitazone are not CYP3A substrates, they can induce the expression of CYP3A enzymes by activation of the nuclear receptors, PXR and CAR [129–132]. Enhanced transcription results in an increase in CYP3A activities and the metabolism of calcineurin inhibitors,

mTOR inhibitors and corticosteroids, increasing the risk of rejection [133]. Immunosuppressant dose adjustment is required to avoid subtherapeutic blood concentrations, and careful monitoring of immunosuppressant blood concentrations is recommended during withdrawal of troglitazone or rosiglitazone and during switching to other antihyperglycemic agent.

4.2.5. *Psychiatric medication*

The most common psychiatric disorders encountered in transplant patients are anxiety, depression, mood disorders, behavior problems, and insomnia that are reversible in most cases; however, they often require psychotherapy with antidepressants, mood stabilizers, anxiolytic agents, or even with antipsychotics. Many of these pharmacons are metabolized by enzymes other than CYP3A4 and do not influence the drug-metabolizing activities of CYP3A4; consequently, metabolic drug interactions with immunosuppressants cannot be expected. Nevertheless, the CYP3A4 inducing or inhibitory properties of some of these psychopharmacons should be considered. The mood stabilizer carbamazepine and valproic acid have been clearly evidenced to be able to activate CAR and PXR. The nuclear receptor activation leads to an increase in transcription of *CYP3A4* gene and CYP3A4 metabolic activity [134, 135], anticipating decrease of immunosuppressant blood concentrations [136]. To reduce the risk of organ rejection, adjustment (increase) of immunosuppressant doses is required with continuous monitoring of immunosuppressant blood levels. Furthermore, the CYP3A4 deinduction process can last for about 2 weeks after cessation of carbamazepine or valproic acid [137]; thus, careful monitoring of immunosuppressant blood concentrations during withdrawal is essential. The comedication with the antidepressant fluvoxamine is contraindicated because of its strong inhibitory properties for CYP3A4 substrates and potential drug interactions with ciclosporin/tacrolimus or with sirolimus/everolimus [80, 138, 139]. For psychotherapeutic agents that are CYP3A substrates (haloperidol, quetiapine, clonazepam, midazolam, alprazolam), continuous monitoring of immunosuppressant blood levels is highly recommended to avoid metabolic drug interactions.

4.2.6. *Treatment of hyperuricemia*

The metabolic drug interactions with ciclosporin/tacrolimus, sirolimus/everolimus, and corticosteroids are generally associated with reversible or irreversible inhibition of CYP3A activities, as well as with transcriptional induction of CYP3A4 and CYP3A5 expression. Clinically significant drug interaction occurs during simultaneous therapy with azathioprine (or 6-mercaptopurine) and allopurinol, the antihyperuricemic agent [140, 141]; however, it involves enzyme other than CYP3As. The metabolism of both 6-mercaptopurine and allopurinol is catalyzed by xanthine oxidase, anticipating metabolic drug interactions and developing serious adverse reactions. As a consequence of inhibition of xanthine oxidase by allopurinol, myelotoxicity is evoked by the accumulation of 6-thioguanine-nucleotide metabolites of azathioprine. The risk of bone marrow depletion is increased in patients with low thiopurine methyl-transferase activity. To avoid the serious myelosuppression during treatment of hyperuricemia and gout, substantial reduction of azathioprine dose (by at least 50%) is required when allopurinol is given concomitantly, or alternative agents other than allopurinol should be considered [142–144].

4.3. Metabolic drug interactions between immunosuppressants and herb components

Pharmacokinetic herb-drug interactions can also significantly influence the outcome of immunosuppressive therapy and long-term graft survival [145]. St John's wort (*Hypericum perforatum*) extract and grapefruit juice are well described as modifiers of pharmacokinetic properties of ciclosporin and tacrolimus [146–148]. St John's wort extract is a herbal product for treatment of symptoms of mild or moderate depression, including anxiety, fatigue, and sleeping problems. The extract contains a number of biologically active components, e.g., hyperforin of high interest. Hyperforin has a strong affinity for PXR and significantly increases the expression and activities of CYP3A4 enzyme, which is involved in metabolism of many drugs [149, 150]. Consequently, chronic consumption of St John's wort extract can decrease the blood concentrations of CYP3A substrates, such as calcineurin inhibitors, mTOR inhibitors, and corticosteroids [151–154]. In addition, St John's wort extract has been reported to induce the expression of ABCB1 transporter that reduces the absorption of ABCB1-ligand drugs from the gut. The hyperforin contents of commercially available St John's wort preparations are variables that appear to significantly affect the extent of pharmacokinetic interactions [150, 155]. Coadministration of ciclosporin with St John's wort extract has been reported to lead a 40–60% decrease of ciclosporin blood concentrations, increasing the risk of rejection; therefore, substantial dose adjustment is required [151, 152, 155–159]. Since clinicians are often unaware of concomitant consumption of herbal supplements, transplant patients should be informed about the drug interaction potential of St John's wort that can endanger the success of organ transplantation.

Concomitant intake of grapefruit (*Citrus paradisi*) or pomelo (*Citrus grandis*) has been demonstrated to increase the bioavailability of immunosuppressants [147, 160, 161]. Some components of these citrus fruits, bergamottin and naringenin responsible for the bitter taste, can inhibit the activities of CYP3A4 and CYP3A5 enzymes both in the intestinal wall and in the liver, resulting in significant reduction of first-pass metabolism of CYP3A substrates, including ciclosporin and tacrolimus [162–164]. Significant reduction of ciclosporin/tacrolimus doses is necessary to avoid the risk of nephrotoxicity or other adverse events associated with immunosuppressive therapy. The furanocoumarin bergamottin is a “suicide substrate,” namely it is metabolized by CYP3A4 to an epoxid metabolite that covalently binds to and inactivates the enzyme [165]. The flavonoid naringenin was found to be a less-potent CYP3A4 inhibitor than bergamottin [166]; however, during consumption of grapefruit, the inhibitory effects of naringenin and bergamottin are added together. Since clear evidence of bergamottin content and CYP3A4 inhibitory potential of citruses other than grapefruit and pomelo was provided [167], the transplantation centers do not recommend citrus consumption for transplant patients during immunosuppressive therapy.

5. Concluding remarks

Although success of organ transplantation is continuously improving, several short- and long-term complications can adversely affect the outcome. One of the most significant factors influencing the long-term graft and patient survival is the appropriate immunosuppressive

therapy. Subtherapeutic blood concentrations of immunosuppressive drugs can evoke acute or chronic graft injury mediated by immunological mechanisms, whereas overdosing leads to over-suppression of the immune system that consequently develops serious infections, as well as adverse and even life-threatening side effects. Because of the narrow therapeutic indexes, dosing of most of the immunosuppressive agents is applied under careful monitoring of their blood concentrations. The knowledge of the potential factors that can modify immunosuppressive therapy, as well as pharmacokinetic and metabolic drug interactions, can decrease the fluctuation of immunosuppressant blood concentrations, can facilitate to avoid the serious adverse events, can improve the therapeutic outcome for transplant patients, and can reduce the medical costs.

The appropriate and tailored immunosuppressive medication is a great challenge and requires careful and continuous attention, because unrecognized simple interactions can induce serious complications. As such during administration of clarithromycin or antifungal agents without dose reduction of calcineurin inhibitors or mTOR inhibitors, blood concentrations of immunosuppressants can substantially exceed the therapeutic range within some days. Without dose modification, a reverse outcome is expected during comedication with anti-convulsants (valproic acid and carbamazepine) or with rifampicin resulting in subtherapeutic blood concentrations of immunosuppressants and increasing the risk of organ rejection. The lack of mycophenolate dose reduction during cessation of ciclosporin or replacement of ciclosporin to another immunosuppressant can also evolve development of serious adverse reactions. It is anticipated that the special attention and the knowledge of potential drug interactions can prevent the majority of misdosing-induced adverse events.

Acknowledgements

This work was supported by MedInProt Synergy VIII program of the Hungarian Academy of Sciences and by the Society of Hungarian Toxicologists.

Conflict of interest

The author declares that there is no conflict of interest.

Author details

Katalin Monostory

Address all correspondence to: monostory.katalin@ttk.mta.hu

Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

References

- [1] Shah RR, Smith RL. Addressing phenoconversion: The Achilles' heel of personalized medicine. *British Journal of Clinical Pharmacology*. 2015;**79**:222-240. DOI: 10.1111/bcp.12441
- [2] Squassina A, Manchia M, Manolopoulos VG, Artac M, Lappa-Manakou C, Karkabouna S, Mitropoulos K, Del Zompo M, Patrinos GP. Realities and expectations of pharmacogenomics and personalized medicine: Impact of translating genetic knowledge into clinical practice. *Pharmacogenomics*. 2010;**11**:1149-1167. DOI: 10.2217/pgs.10.97
- [3] Gervasini G, Benítez J, Carrillo JA. Pharmacogenetic testing and therapeutic drug monitoring are complementary tools for optimal individualization of drug therapy. *European Journal of Clinical Pharmacology*. 2010;**66**:755-774. DOI: 10.1007/s00228-010-0857-7
- [4] Shah RR, Shah DR. Personalized medicine: Is it a pharmacogenetic mirage? *British Journal of Clinical Pharmacology*. 2012;**74**:698-721. DOI: 10.1111/j.1365-2125.2012.04328.x
- [5] Sim SC, Kacevska M, Ingelman-Sundberg M. Pharmacogenomics of drug-metabolizing enzymes: A recent update on clinical implications and endogenous effects. *The Pharmacogenomics Journal*. 2013;**13**:1-11. DOI: 10.1038/tpj.2012.45
- [6] Zhou SF, Liu JP, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. *Drug Metabolism Reviews*. 2009;**41**:89-295. DOI: 10.1021/tx0100439
- [7] Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacology & Therapeutics*. 2013;**138**:103-141. DOI: 10.1016/j.pharmthera.2012.12.007
- [8] Samer CF, Lorenzini KI, Rollason V, Daali Y, Desmeules JA. Applications of CYP450 testing in the clinical setting. *Molecular Diagnosis & Therapy*. 2013;**17**:165-184. DOI: 10.1007/s40291-013-0028-5
- [9] Zhou ZW, Chen XW, Sneed KB, Yang YX, Zhang X, He ZX, Chow K, Yang T, Duan W, Zhou SF. Clinical association between pharmacogenomics and adverse drug reactions. *Drugs*. 2015;**75**:589-631. DOI: 10.1007/s40265-015-0375-0
- [10] Rendic S, Guengerich FP. Update information on drug metabolism systems - 2009, part II: Summary of information on the effects of diseases and environmental factors on human cytochrome P450 (CYP) enzymes and transporters. *Current Drug Metabolism*. 2010;**11**:4-84. DOI: 10.2174/138920010791110917
- [11] Shah RR, Gaedigk A, LLerena A, Eichelbaum M, Stingl J, Smith RL. CYP450 genotype and pharmacogenetic association studies: A critical appraisal. *Pharmacogenomics*. 2016;**17**:259-275. DOI: 10.2217/pgs.15.172
- [12] Zhou Y, Ingelman-Sundberg M, Lauschke VM. Worldwide distribution of cytochrome P450 alleles: A meta-analysis of population-scale sequencing projects. *Clinical Pharmacology and Therapeutics*. 2017;**102**:688-700. DOI: 10.1002/cpt.690

- [13] Temesvári M, Kóbori L, Paulik J, Sárváry E, Belic A, Monostory K. Estimation of drug-metabolizing capacity by cytochrome P450 genotyping and expression. *The Journal of Pharmacology and Experimental Therapeutics*. 2012;**341**:294-305. DOI: 10.1124/jpet.111.189597
- [14] Amirimani B, Ning B, Deitz AC, Weber BL, Kadlubar FF, Rebbeck TR. Increased transcriptional activity of the *CYP3A4*1B* promoter variant. *Environmental and Molecular Mutagenesis*. 2003;**42**:299-305. DOI: 10.1002/em.10199
- [15] García-Martín E, Martínez C, Pizarro RM, García-Gamito FJ, Gullsten H, Raunio H, Agúndez JA. *CYP3A4* variant alleles in white individuals with low *CYP3A4* enzyme activity. *Clinical Pharmacology and Therapeutics*. 2002;**71**:196-204. DOI: 10.1067/mcp.2002.121371
- [16] Okubo M, Murayama N, Shimizu M, Shimada T, Guengerich FP, Yamazaki H. *CYP3A4* intron 6 C>T polymorphism (*CYP3A4*22*) is associated with reduced *CYP3A4* protein level and function in human liver microsomes. *The Journal of Toxicological Sciences*. 2013;**38**:349-354. DOI: 10.2131/jts.38.349
- [17] Zhou SF. Drugs behave as substrates, inhibitors and inducers of human cytochrome P450 3A4. *Current Drug Metabolism*. 2008;**9**:310-322. DOI: 10.2174/138920008784220664
- [18] Monostory K, Dvorak Z. Steroid regulation of drug-metabolizing cytochromes P450. *Current Drug Metabolism*. 2011;**12**:154-172. DOI: 10.2174/138920011795016854
- [19] Hukkanen J. Induction of cytochrome P450 enzymes: A view on human in vivo findings. *Expert Review of Clinical Pharmacology*. 2012;**5**:569-585. DOI: 10.1586/ecp.12.39
- [20] Sinz MW. Evaluation of pregnane X receptor (PXR)-mediated *CYP3A4* drug-drug interactions in drug development. *Drug Metabolism Reviews*. 2013;**45**:3-14. DOI: 10.3109/03602532.2012.743560
- [21] Matoulková P, Pávek P, Malý J, Vlček J. Cytochrome P450 enzyme regulation by glucocorticoids and consequences in terms of drug interaction. *Expert Opinion on Drug Metabolism & Toxicology*. 2014;**10**:425-435. DOI: 10.1517/17425255.2014.878703
- [22] Aitken AE, Morgan ET. Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. *Drug Metabolism and Disposition*. 2007;**35**:1687-1693. DOI: 10.1124/dmd.107.015511
- [23] Dickmann LJ, Patel SK, Rock DA, Wienkers LC, Slatter JG. Effects of interleukin-6 (IL-6) and an anti-IL-6 monoclonal antibody on drug-metabolizing enzymes in human hepatocyte culture. *Drug Metabolism and Disposition*. 2011;**39**:1415-1422. DOI: 10.1124/dmd.111.038679
- [24] Dickmann LJ, Patel SK, Wienkers LC, Slatter JG. Effects of interleukin 1 β (IL-1 β) and IL-1 β /interleukin 6 (IL-6) combinations on drug metabolizing enzymes in human hepatocyte culture. *Current Drug Metabolism*. 2012;**13**:930-937. DOI: 10.2174/138920012802138642

- [25] Ning R, Zhan Y, He S, Hu Y, Zhu Z, Hu G, Yan B, Yang J, Liu W. Interleukin-6 induces DEC1, promotes DEC1 interaction with RXR α and suppresses the expression of PXR, CAR and their target genes. *Frontiers in Pharmacology*. 2017;**8**:866. DOI: 10.3389/fphar.2017.00866
- [26] Shah RR, Smith RL. Inflammation-induced phenoconversion of polymorphic drug metabolizing enzymes: Hypothesis with implications for personalized medicine. *Drug Metabolism and Disposition*. 2015;**43**:400-410. DOI: 10.1124/dmd.114.061093
- [27] Shah RR. Pharmacogenetics and precision medicine: Is inflammation a covert threat to effective genotype-based therapy? *Therapeutic Advances in Drug Safety*. 2017;**8**:267-272. DOI: 10.1177/2042098617712657
- [28] Ryman JT, Meibohm B. Pharmacokinetics of monoclonal antibodies. *CPT: Pharmacometrics & Systems Pharmacology*. 2017;**6**:576-588. DOI: 10.1002/psp4.12224
- [29] Penninga L, Wettergren A, Chan AW, Steinbrüchel DA, Gluud C. Calcineurin inhibitor minimisation versus continuation of calcineurin inhibitor treatment for liver transplant recipients. *Cochrane Database of Systematic Reviews*. 2012;**3**:CD008852. DOI: 10.1002/14651858.CD008852.pub2
- [30] Choudhary NS, Saigal S, Shukla R, Kotecha H, Saraf N, Soin AS. Current status of immunosuppression in liver transplantation. *Journal of Clinical and Experimental Hepatology*. 2013;**3**:150-158. DOI: 10.1016/j.jceh.2013.04.005
- [31] Azzi JR, Sayegh MH, Mallat SG. Calcineurin inhibitors: 40 years later, can't live without. *Journal of Immunology*. 2013;**191**:5785-5791. DOI: 10.4049/jimmunol.1390055
- [32] Christians U, Sewing KF. Cyclosporin metabolism in transplant patients. *Pharmacology & Therapeutics*. 1993;**57**:291-345. DOI: 10.1016/0163-7258(93)90059-M
- [33] Christians U, Kohlhaw K, Budniak J, Bleck JS, Schottmann R, Schlitt HJ, Almeida VM, Deters M, Wonigeit K, Pichlmayr R, Sewing KF. Cyclosporin metabolite pattern in blood and urine of liver graft recipients. I. Association of cyclosporin metabolites with nephrotoxicity. *European Journal of Clinical Pharmacology*. 1991;**41**:285-290
- [34] Christians U, Kohlhaw K, Sürig T, Bader A, Schottmann R, Linck A, Ringe B, Sewing KF. Parallel blood concentrations of second-generation cyclosporine metabolites and bilirubin in liver graft recipients. *Therapeutic Drug Monitoring*. 1995;**17**:487-498. DOI: 10.1097/00007691-199510000-00009
- [35] Monostory K, Tóth K, Kiss Á, Háfra E, Csikány N, Paulik J, Sárváry E, Kóbori L. Personalizing calcineurin inhibitor therapy by adjusting to donor CYP3A-status in liver transplant patients. *British Journal of Clinical Pharmacology*. 2015;**80**:1429-1437. DOI: 10.1111/bcp.12747
- [36] Iwasaki K. Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. *Drug Metabolism and Pharmacokinetics*. 2007;**22**:328-335. DOI: 10.2133/dmpk.22.328

- [37] Iwasaki K, Shiraga T, Nagase K, Tozuka Z, Noda K, Sakuma S, Fujitsu T, Shimatani K, Sato A, Fujioka M. Isolation, identification, and biological activities of oxidative metabolites of FK506, a potent immunosuppressive macrolide lactone. *Drug Metabolism and Disposition*. 1993;**21**:971-977
- [38] Iwasaki K, Shiraga T, Matsuda H, Nagase K, Tokuma Y, Hata T, Fujii Y, Sakuma S, Fujitsu T, Fujikawa A, Shimatani K, Sato A, Fujioka M. Further metabolism of FK506 (tacrolimus). Identification and biological activities of the metabolites oxidized at multiple sites of FK506. *Drug Metabolism and Disposition*. 1995;**23**:28-34
- [39] Zegarska J, Hryniewiecka E, Zochowska D, Samborowska E, Jazwiec R, Borowiec A, Tszysznick W, Chmura A, Nazarewski S, Dadlez M, Paczek L. Tacrolimus metabolite M-III may have nephrotoxic and myelotoxic effects and increase the incidence of infections in kidney transplant recipients. *Transplantation Proceedings*. 2016;**48**:1539-1542. DOI: 10.1016/j.transproceed.2015.12.133
- [40] Kamdem LK, Streit F, Zanger UM, Brockmöller J, Oellerich M, Armstrong VW, Wojnowski L. Contribution of CYP3A5 to the in vitro hepatic clearance of tacrolimus. *Clinical Chemistry*. 2005;**51**:1374-1381. DOI: 10.1373/clinchem.2005.050047
- [41] Provenzani A, Notarbartolo M, Labbozzetta M, Poma P, Vizzini G, Salis P, Caccamo C, Bertani T, Palazzo U, Polidori P, Gridelli B, D'Alessandro N. Influence of CYP3A5 and ABCB1 gene polymorphisms and other factors on tacrolimus dosing in Caucasian liver and kidney transplant patients. *International Journal of Molecular Medicine*. 2011;**28**: 1093-1102. DOI: 10.3892/ijmm.2011.794
- [42] Moes DJ, Guchelaar HJ, de Fijter JW. Sirolimus and everolimus in kidney transplantation. *Drug Discovery Today*. 2015;**20**:1243-1249. DOI: 10.1016/j.drudis.2015.05.006
- [43] Jacquet A, Francois H, Frangie C, Ahmad L, Charpentier B, Durrbach A. Prevention of calcineurin inhibitor nephrotoxicity in renal transplantation. *Transplant Immunology*. 2008;**20**:29-31. DOI: 10.1016/j.trim.2008.09.002
- [44] Kacar S, Gurkan A, Karaca C, Varılsuha C, Tilif S. Low-dose calcineurin inhibitor regimen combined with mammalian target of rapamycin inhibitors preserves kidney functions in renal transplant recipients without allograft nephropathy. *Transplantation Proceedings*. 2010;**42**:3513-3516. DOI: 10.1016/j.transproceed.2010.08.043
- [45] Shihab F, Christians U, Smith L, Wellen JR, Kaplan B. Focus on mTOR inhibitors and tacrolimus in renal transplantation: Pharmacokinetics, exposure-response relationships, and clinical outcomes. *Transplant Immunology*. 2014;**31**:22-32. DOI: 10.1016/j.trim.2014.05.002
- [46] Jacobsen W, Serkova N, Hausen B, Morris RE, Benet LZ, Christians U. Comparison of the in vitro metabolism of the macrolide immunosuppressants sirolimus and RAD. *Transplantation Proceedings*. 2001;**33**:514-515. DOI: 10.1016/S0041-1345(00)02116-3
- [47] Mahalati K, Kahan BD. Clinical pharmacokinetics of sirolimus. *Clinical Pharmacokinetics*. 2001;**40**:573-585. DOI: 10.2165/00003088-200140080-00002
- [48] Maltzman JS, Koretzky GA. Azathioprine: Old drug, new actions. *The Journal of Clinical Investigation*. 2003;**111**:1122-1224. DOI: 10.1172/JCI18384

- [49] Rowland K, Lennard L, Lilleyman JS. In vitro metabolism of 6-mercaptopurine by human liver cytosol. *Xenobiotica*. 1999;**29**:615-628. DOI: 10.1080/004982599238434
- [50] Relling MV, Gardner EE, Sandborn WJ, Schmiegelow K, Pui CH, Yee SW, Stein CM, Carrillo M, Evans WE, Hicks JK, Schwab M, Klein TE. Clinical Pharmacogenetics implementation consortium. Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. *Clinical Pharmacology and Therapeutics*. 2013;**93**:324-325. DOI: 10.1038/clpt.2013.4
- [51] Staatz CE, Tett SE. Pharmacology and toxicology of mycophenolate in organ transplant recipients: An update. *Archives of Toxicology*. 2014;**88**:1352-1389. DOI: 10.1007/s00204-014-1247-1
- [52] Picard N, Ratanasavanh D, Prémaud A, Le Meur Y, Marquet P. Identification of the UDP-glucuronosyltransferase isoforms involved in mycophenolic acid phase II metabolism. *Drug Metabolism and Disposition*. 2005;**33**:139-146. DOI: 10.1124/dmd.104.001651
- [53] Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of mycophenolate in patients with autoimmune disease. *Clinical Pharmacokinetics*. 2007;**46**:13-58. DOI: 10.2165/00003088-200746010-00002
- [54] Lamba V, Sangkuhl K, Sanghavi K, Fish A, Altman RB, Klein TE. PharmGKB summary: Mycophenolic acid pathway. *Pharmacogenetics and Genomics*. 2014;**24**:73-79. DOI: 10.1097/FPC.0000000000000010
- [55] Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. *The New England Journal of Medicine*. 2005;**353**:1711-1723. DOI: 10.1056/NEJMr050541
- [56] Pichard L, Fabre I, Daujat M, Domergue J, Joyeux H, Maurel P. Effect of corticosteroids on the expression of cytochromes P450 and on cyclosporin a oxidase activity in primary cultures of human hepatocytes. *Molecular Pharmacology*. 1992;**41**:1047-1055
- [57] Pascussi JM, Gerbal-Chaloin S, Drocourt L, Maurel P, Vilarem MJ. The expression of CYP2B6, CYP2C9 and CYP3A4 genes: A tangle of networks of nuclear and steroid receptors. *Biochimica et Biophysica Acta*. 2003;**1619**:243-253. DOI: 10.1016/S0304-4165(02)00483-X
- [58] Wong TC, Lo CM, Fung JY. Emerging drugs for prevention of T-cell mediated rejection in liver and kidney transplantation. *Expert Opinion on Emerging Drugs*. 2017;**22**:123-136. DOI: 10.1080/14728214.2017.1330884
- [59] Kuglstatter A, Mueller F, Kusznir E, Gsell B, Stihle M, Thoma R, Benz J, Aspeslet L, Freitag D, Hennig M. Structural basis for the cyclophilin a binding affinity and immunosuppressive potency of E-ISA247 (voclosporin). *Acta Crystallographica. Section D, Biological Crystallography*. 2011;**67**:119-123. DOI: 10.1107/S0907444910051905
- [60] Ling SY, Huizinga RB, Mayo PR, Larouche R, Freitag DG, Aspeslet LJ, Foster RT. Cytochrome P450 3A and P-glycoprotein drug-drug interactions with voclosporin. *British Journal of Clinical Pharmacology*. 2014;**77**:1039-1050. DOI: 10.1111/bcp.12309

- [61] Tedesco-Silva H, Kho MM, Hartmann A, Vitko S, Russ G, Rostaing L, Budde K, Campistol JM, Eris J, Krishnan I, Gopalakrishnan U, Klupp J. Sotrastaurin in calcineurin inhibitor-free regimen using everolimus in de novo kidney transplant recipients. *American Journal of Transplantation*. 2013;**13**:1757-1768. DOI: 10.1111/ajt.12255
- [62] Pascher A, DeSimone P, Pratschke J, Salamé E, Pirenne J, Isonemi H, Bijarnia M, Krishnan I, Klupp J. Protein kinase C inhibitor sotrastaurin in de novo liver transplant recipients: A randomized phase II trial. *American Journal of Transplantation*. 2015;**15**:1283-1292. DOI: 10.1111/ajt.13175
- [63] van Gelder T, Fisher L, Shihab F, Shipkova M. Optimizing everolimus exposure when combined with calcineurin inhibitors in solid organ transplantation. *Transplantation Reviews (Orlando, Fla.)*. 2017;**31**:151-157. DOI: 10.1016/j.trre.2017.02.007
- [64] Sharif A, Shabir S, Chand S, Cockwell P, Ball S, Borrows R. Meta-analysis of calcineurin-inhibitor-sparing regimens in kidney transplantation. *Journal of the American Society of Nephrology*. 2011;**22**:2107-2118. DOI: 10.1681/ASN.2010111160
- [65] Martin P, Riley R, Back DJ, Owen A. Comparison of the induction profile for drug disposition proteins by typical nuclear receptor activators in human hepatic and intestinal cells. *British Journal of Pharmacology*. 2008;**153**:805-819. DOI: 10.1038/sj.bjp.0707601
- [66] Hesselink DA, Ngyuen H, Wabbijn M, Gregoor PJ, Steyerberg EW, van Riemsdijk IC, Weimar W, van Gelder T. Tacrolimus dose requirement in renal transplant recipients is significantly higher when used in combination with corticosteroids. *British Journal of Clinical Pharmacology*. 2003;**56**:327-330. DOI: 10.1046/j.0306-5251.2003.01882.x
- [67] Anglicheau D, Flamant M, Schlageter MH, Martinez F, Cassinat B, Beaune P, Legendre C, Thervet E. Pharmacokinetic interaction between corticosteroids and tacrolimus after renal transplantation. *Nephrology, Dialysis, Transplantation*. 2003;**18**:2409-2414. DOI: 10.1093/ndt/gfg381
- [68] van Duijnhoven EM, Boots JM, Christiaans MH, Stolk LM, Undre NA, van Hooff JP. Increase in tacrolimus trough levels after steroid withdrawal. *Transplant International*. 2003;**16**:721-725. DOI: 10.1007/s00147-003-0615-1
- [69] Roberts PJ, Rollins KD, Kashuba AD, Paine MF, Nelsen AC, Williams EE, Moran C, Lamba JK, Schuetz EG, Hawke RL. The influence of CYP3A5 genotype on dexamethasone induction of CYP3A activity in African Americans. *Drug Metabolism and Disposition*. 2008;**36**:1465-1469. DOI: 10.1124/dmd.107.020065
- [70] Naito T, Shinno K, Maeda T, Kagawa Y, Hashimoto H, Otsuka A, Takayama T, Ushiyama T, Suzuki K, Ozono S. Effects of calcineurin inhibitors on pharmacokinetics of mycophenolic acid and its glucuronide metabolite during the maintenance period following renal transplantation. *Biological & Pharmaceutical Bulletin*. 2006;**29**:275-280. DOI: 10.1248/bpb.29.275
- [71] Cattaneo D, Merlini S, Zenoni S, Baldelli S, Gotti E, Remuzzi G, Perico N. Influence of co-medication with sirolimus or cyclosporine on mycophenolic acid pharmacokinetics in kidney transplantation. *American Journal of Transplantation*. 2005;**5**:2937-2944. DOI: 10.1111/j.1600-6143.2005.01107.x

- [72] Dalal P, Shah G, Chhabra D, Gallon L. Role of tacrolimus combination therapy with mycophenolate mofetil in the prevention of organ rejection in kidney transplant patients. *International Journal of Nephrology and Renovascular Disease*. 2010;**3**:107-115. DOI: 10.2147/IJNRD.S7044
- [73] Kovarik JM, Beyer D, Schmouder RL. Everolimus drug interactions: Application of a classification system for clinical decision making. *Biopharmaceutics & Drug Disposition*. 2006;**27**:421-426. DOI: 10.1002/bdd.524
- [74] Lu C, Hatsis P, Berg C, Lee FW, Balani SK. Prediction of pharmacokinetic drug-drug interactions using human hepatocyte suspension in plasma and cytochrome P450 phenotypic data. II. In vitro-in vivo correlation with ketoconazole. *Drug Metabolism and Disposition*. 2008;**36**:1255-1260. DOI: 10.1124/dmd.107.018796
- [75] Lu C, Berg C, Prakash SR, Lee FW, Balani SK. Prediction of pharmacokinetic drug-drug interactions using human hepatocyte suspension in plasma and cytochrome P450 phenotypic data. III. In vitro-in vivo correlation with fluconazole. *Drug Metabolism and Disposition*. 2008;**36**:1261-1266. DOI: 10.1124/dmd.107.019000
- [76] Yamazaki H, Nakamoto M, Shimizu M, Murayama N, Niwa T. Potential impact of cytochrome P450 3A5 in human liver on drug interactions with triazoles. *British Journal of Clinical Pharmacology*. 2010;**69**:593-597. DOI: 10.1111/j.1365-2125.2010.03656.x
- [77] Zhang S, Pillai VC, Mada SR, Strom S, Venkataramanan R. Effect of voriconazole and other azole antifungal agents on CYP3A activity and metabolism of tacrolimus in human liver microsomes. *Xenobiotica*. 2012;**42**:409-416. DOI: 10.3109/00498254.2011.631224
- [78] Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. *Pharmacotherapy*. 2006;**26**:1730-1744. DOI: 10.1592/phco.26.12.1730
- [79] Galetin A, Burt H, Gibbons L, Houston JB. Prediction of time-dependent CYP3A4 drug-drug interactions: Impact of enzyme degradation, parallel elimination pathways, and intestinal inhibition. *Drug Metabolism and Disposition*. 2006;**34**:166-175. DOI: 10.1124/dmd/105.006874
- [80] Watanabe A, Nakamura K, Okudaira N, Okazaki O, Sudo K. Risk assessment for drug-drug interaction caused by metabolism-based inhibition of CYP3A using automated in vitro assay systems and its application in the early drug discovery process. *Drug Metabolism and Disposition*. 2007;**35**:1232-1238. DOI: 10.1124/dmd.107.015016
- [81] Aueviriyavit S, Kobayashi K, Chiba K. Species differences in mechanism-based inactivation of CYP3A in humans, rats and mice. *Drug Metabolism and Pharmacokinetics*. 2010;**25**:93-100. DOI: 10.2133/dmpk.25.93
- [82] Kosaka M, Kosugi Y, Hirabayashi H. Risk assessment using cytochrome P450 time-dependent inhibition assays at single time and concentration in the early stage of drug discovery. *Journal of Pharmaceutical Sciences*. 2017;**106**:2839-2846. DOI: 10.1016/j.xphs.2017.04.077
- [83] Westphal JF. Macrolide-induced clinically relevant drug interactions with cytochrome P-450A (CYP) 3A4: An update focused on clarithromycin, azithromycin and dirithromycin. *British Journal of Clinical Pharmacology*. 2000;**50**:285-295. DOI: 10.1046/j.1365-2125.2000.00261.x

- [84] Rubinstein E. Comparative safety of the different macrolides. *International Journal of Antimicrobial Agents*. 2001;**18**(Suppl 1):S71-S76. DOI: 10.1016/S0924-8579(01)00397-1
- [85] Capone D, Palmiero G, Gentile A, Basile V, Federico S, Sabbatini M, Potenza M, Perfetti A, Pieri M, Tarantino G. A pharmacokinetic interaction between clarithromycin and sirolimus in kidney transplant recipient. *Current Drug Metabolism*. 2007;**8**:379-381. DOI: 10.2174/138920007780655405
- [86] Pea F, Cojutti P, Tursi V, Livi U, Baraldo M. Everolimus overexposure in a heart transplant patient receiving clarithromycin for the treatment of pneumonia. *Transplant Infectious Disease*. 2015;**17**:926-928. DOI: 10.1111/tid.12446
- [87] Page RL 2nd, Ruscin JM, Fish D, Lapointe M. Possible interaction between intravenous azithromycin and oral cyclosporine. *Pharmacotherapy*. 2001;**21**:1436-1443. DOI: 10.1592/phco.21.17.1436.34434
- [88] Mori T, Aisa Y, Nakazato T, Yamazaki R, Ikeda Y, Okamoto S. Tacrolimus-azithromycin interaction in a recipient of allogeneic bone marrow transplantation. *Transplant International*. 2005;**18**:757-758. DOI: 10.1111/j.1432-2277.2005.00135.x
- [89] Polasek TM, Miners JO. Quantitative prediction of macrolide drug-drug interaction potential from in vitro studies using testosterone as the human cytochrome P4503A substrate. *European Journal of Clinical Pharmacology*. 2006;**62**:203-208. DOI: 10.1007/s00228-005-0091-x
- [90] Monostory K, Pascussi J-M. Regulation of drug-metabolizing human cytochrome P450s. *Acta Chimica Slovenica*. 2008;**55**:20-37
- [91] Hebert MF, Fisher RM, Marsh CL, Dressler D, Bekersky I. Effects of rifampin on tacrolimus pharmacokinetics in healthy volunteers. *Journal of Clinical Pharmacology*. 1999;**39**:91-96. DOI: 10.1177/00912709922007499
- [92] Bhaloo S, Prasad GV. Severe reduction in tacrolimus levels with rifampin despite multiple cytochrome P450 inhibitors: A case report. *Transplantation Proceedings*. 2003;**35**:2449-2451. DOI: 10.1016/j.transproceed.2003.08.019
- [93] Lee YT, Hwang S, Lee SG, Kim KW, Choi NK, Park GC, Yu YD, Yoo JW, Kim WS, Shim TS. Living-donor liver transplantation in patients with concurrent active tuberculosis at transplantation. *The International Journal of Tuberculosis and Lung Disease*. 2010;**14**:1039-1044
- [94] Gimenez F, Foeillet E, Bourdon O, Weller S, Garret C, Bidault R, Singlas E. Evaluation of pharmacokinetic interactions after oral administration of mycophenolate mofetil and valaciclovir or aciclovir to healthy subjects. *Clinical Pharmacokinetics*. 2004;**43**:685-692. DOI: 10.2165/00003088-200443100-00004
- [95] Brum S, Nolasco F, Sousa J, Ferreira A, Possante M, Pinto JR, Barroso E, Santos JR. Leukopenia in kidney transplant patients with the association of valganciclovir and mycophenolate mofetil. *Transplantation Proceedings*. 2008;**40**:752-754. DOI: 10.1016/j.transproceed.2008.02.048

- [96] Mohammadpoor AH, Nazemian F, Khayyat MH, Naghibi M, Bahrami A, Kazemi M. Effect of ganciclovir on pharmacokinetics of mycophenolic mofetil, in kidney transplant patients. *Iranian Journal of Basic Medical Sciences*. 2008;**10**:233-238
- [97] Sheikh AM, Wolf DC, Lebovics E, Goldberg R, Horowitz HW. Concomitant human immunodeficiency virus protease inhibitor therapy markedly reduces tacrolimus metabolism and increases blood levels. *Transplantation*. 1999;**68**:307-309. DOI: 10.1097/00007890-199907270-00027
- [98] Vogel M, Voigt E, Michaelis HC, Sudhop T, Wolff M, Türler A, Sauerbruch T, Rockstroh JK, Spengler U. Management of drug-to-drug interactions between cyclosporine a and the protease-inhibitor lopinavir/ritonavir in liver-transplanted HIV-infected patients. *Liver Transplantation*. 2004;**10**:939-944. DOI: 10.1002/lt.20165
- [99] Teicher E, Vincent I, Bonhomme-Faivre L, Abbara C, Barrail A, Boissonnas A, Duclos-Vallée JC, Taburet AM, Samuel D, Vittecoq D. Effect of highly active antiretroviral therapy on tacrolimus pharmacokinetics in hepatitis C virus and HIV co-infected liver transplant recipients in the ANRS HC-08 study. *Clinical Pharmacokinetics*. 2007;**46**:941-952. DOI: 10.2165/00003088-200746110-00002
- [100] Mertz D, Battegay M, Marzolini C, Mayr M. Drug-drug interaction in a kidney transplant recipient receiving HIV salvage therapy and tacrolimus. *American Journal of Kidney Diseases*. 2009;**54**:e1-e4. DOI: 10.1053/j.ajkd.2009.01.268
- [101] Pulzer A, Seybold U, Schönermarck U, Stangl M, Habicht A, Bogner JR, Franke J, Fischereder M. Calcineurin inhibitor dose-finding before kidney transplantation in HIV patients. *Transplant International*. 2013;**26**:254-258. DOI: 10.1111/tri.12020
- [102] Mathis AS, Davé N, Knipp GT, Friedman GS. Drug-related dyslipidemia after renal transplantation. *American Journal of Health-System Pharmacy*. 2004;**61**:565-585
- [103] Christians U, Jacobsen W, Floren LC. Metabolism and drug interactions of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors in transplant patients: Are the statins mechanistically similar? *Pharmacology & Therapeutics*. 1998;**80**:1-34. DOI: 10.1016/S0163-7258(98)00016-3
- [104] Asberg A. Interactions between cyclosporin and lipid-lowering drugs: Implications for organ transplant recipients. *Drugs*. 2003;**63**:367-378. DOI: 10.2165/00003495-200363040-00003
- [105] Neuvonen PJ, Backman JT, Niemi M. Pharmacokinetic comparison of the potential over-the-counter statins simvastatin, lovastatin, fluvastatin and pravastatin. *Clinical Pharmacokinetics*. 2008;**47**:463-474. DOI: 10.2165/00003088-200847070-00003
- [106] Causevic-Ramosevac A, Semiz S. Drug interactions with statins. *Acta Pharmaceutica*. 2013;**63**:277-293. DOI: 10.2478/acph-2013-0022
- [107] Gertz M, Cartwright CM, Hobbs MJ, Kenworthy KE, Rowland M, Houston JB, Galetin A. Cyclosporine inhibition of hepatic and intestinal CYP3A4, uptake and efflux transporters: Application of PBPK modeling in the assessment of drug-drug interaction potential. *Pharmaceutical Research*. 2013;**30**:761-780. DOI: 10.1007/s11095-012-0918-y

- [108] Christians U, Jacobsen W, Benet LZ, Lampen A. Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clinical Pharmacokinetics*. 2002;**41**:813-851. DOI: 10.2165/00003088-200241110-00003
- [109] Lemahieu WP, Hermann M, Asberg A, Verbeke K, Holdaas H, Vanrenterghem Y, Maes BD. Combined therapy with atorvastatin and calcineurin inhibitors: No interactions with tacrolimus. *American Journal of Transplantation*. 2005;**5**:2236-2243. DOI: 10.1111/j.1600-6143.2005.01005.x
- [110] Ma B, Prueksaritanont T, Lin JH. Drug interactions with calcium channel blockers: Possible involvement of metabolite-intermediate complexation with CYP3A. *Drug Metabolism and Disposition*. 2000;**28**:125-130
- [111] Wang YH, Jones DR, Hall SD. Prediction of cytochrome P450 3A inhibition by verapamil enantiomers and their metabolites. *Drug Metabolism and Disposition*. 2004;**32**:259-266. DOI: 10.1124/dmd.32.2.259
- [112] Wang YH, Jones DR, Hall SD. Differential mechanism-based inhibition of CYP3A4 and CYP3A5 by verapamil. *Drug Metabolism and Disposition*. 2005;**33**:664-671. DOI: 10.1124/dmd/104.001834
- [113] Zhao P, Lee CA, Kunze KL. Sequential metabolism is responsible for diltiazem-induced time-dependent loss of CYP3A. *Drug Metabolism and Disposition*. 2007;**35**:704-712. DOI: 10.1124/dmd.106.013847
- [114] Krasulova K, Holas O, Anzenbacher P. Influence of amlodipine enantiomers on human microsomal cytochromes P450: Stereoselective time-dependent inhibition of CYP3A enzyme activity. *Molecules*. 2017;**22**:E1879. DOI: 10.3390/molecules22111879
- [115] Crocker JF, Renton KW, LeVatte TL, McLellan DH. The interaction of the calcium channel blockers verapamil and nifedipine with cyclosporin a in pediatric renal transplant patients. *Pediatric Nephrology*. 1994;**8**:408-411. DOI: 10.1007/BF00856514
- [116] Campana C, Regazzi MB, Buggia I, Molinaro M. Clinically significant drug interactions with cyclosporin. An update. *Clinical Pharmacokinetics*. 1996;**30**:141-179. DOI: 10.2165/00003088-199630020-00004
- [117] Yildiz A, Sever MS, Türkmen A, Ecder T, Türk S, Akkaya V, Ark E. Interaction between cyclosporine a and verapamil, felodipine, and isradipine. *Nephron*. 1999;**81**:117-118
- [118] Hebert MF, Lam AY. Diltiazem increases tacrolimus concentrations. *The Annals of Pharmacotherapy*. 1999;**33**:680-682. DOI: 10.1345/aph.18356
- [119] Böttiger Y, Säwe J, Brattström C, Tollemar J, Burke JT, Häss G, Zimmerman JJ. Pharmacokinetic interaction between single oral doses of diltiazem and sirolimus in healthy volunteers. *Clinical Pharmacology and Therapeutics*. 2001;**69**:32-40. DOI: 10.1067/mcp.2001.112513
- [120] Jones TE, Morris RG. Pharmacokinetic interaction between tacrolimus and diltiazem: Dose-response relationship in kidney and liver transplant recipients. *Clinical Pharmacokinetics*. 2002;**41**:381-388

- [121] Kovarik JM, Beyer D, Bizot MN, Jiang Q, Allison MJ, Schmouder RL. Pharmacokinetic interaction between verapamil and everolimus in healthy subjects. *British Journal of Clinical Pharmacology*. 2005;**60**:434-437. DOI: 10.1111/j.1365-2125.2005.02434.x
- [122] Bernard E, Goutelle S, Bertrand Y, Bleyzac N. Pharmacokinetic drug-drug interaction of calcium channel blockers with cyclosporine in hematopoietic stem cell transplant children. *The Annals of Pharmacotherapy*. 2014;**48**:1580-1584. DOI: 10.1177/1060028014550644
- [123] Zimmerman JJ. Exposure-response relationships and drug interactions of sirolimus. *The AAPS Journal*. 2004;**6**:e28. DOI: 10.1208/aapsj060428
- [124] Zuo XC, Zhou YN, Zhang BK, Yang GP, Cheng ZN, Yuan H, Ouyang DS, Liu SK, Barrett JS, Li PJ, Liu Z, Tan HY, Guo R, Zhou LY, Xie YL, Li ZJ, Li J, Wang CJ, Wang JL. Effect of CYP3A5*3 polymorphism on pharmacokinetic drug interaction between tacrolimus and amlodipine. *Drug Metabolism and Pharmacokinetics*. 2013;**28**:398-405. DOI: 10.2133/dmpk.DMPK-12-RG-148
- [125] Kaijser M, Johnsson C, Zezina L, Backman U, Dimény E, Fellström B. Elevation of cyclosporin a blood levels during carvedilol treatment in renal transplant patients. *Clinical Transplantation*. 1997;**11**:577-581
- [126] Bader FM, Hagan ME, Crompton JA, Gilbert EM. The effect of beta-blocker use on cyclosporine level in cardiac transplant recipients. *The Journal of Heart and Lung Transplantation*. 2005;**24**:2144-2147. DOI: 10.1016/j.healun.2005.05.002
- [127] Oldham HG, Clarke SE. Vitro identification of the human cytochrome P450 enzymes involved in the metabolism of R(+)- and S(-)-carvedilol. *Drug Metabolism and Disposition*. 1997;**25**:970-977
- [128] Amioka K, Kuzuya T, Kushiara H, Ejiri M, Nitta A, Nabeshima T. Carvedilol increases ciclosporin bioavailability by inhibiting P-glycoprotein-mediated transport. *The Journal of Pharmacy and Pharmacology*. 2007;**59**:1383-1387. DOI: 10.1211/jpp.59.10.0008
- [129] Ramachandran V, Kostrubsky VE, Komoroski BJ, Zhang S, Dorko K, Esplen JE, Strom SC, Venkataramanan R. Troglitazone increases cytochrome P-450 3A protein and activity in primary cultures of human hepatocytes. *Drug Metabolism and Disposition*. 1999;**27**:1194-1199
- [130] Sahi J, Black CB, Hamilton GA, Zheng X, Jolley S, Rose KA, Gilbert D, LeCluyse EL, Sinz MW. Comparative effects of thiazolidinediones on in vitro P450 enzyme induction and inhibition. *Drug Metabolism and Disposition*. 2003;**31**:439-446. DOI: 10.1124/dmd.31.4.439
- [131] Sinz M, Kim S, Zhu Z, Chen T, Anthony M, Dickinson K, Rodrigues AD. Evaluation of 170 xenobiotics as transactivators of human pregnane X receptor (hPXR) and correlation to known CYP3A4 drug interactions. *Current Drug Metabolism*. 2006;**7**:375-388. DOI: 10.2174/138920006776873535
- [132] Elbarbry FA, Marfleet T, Shoker AS. Drug-drug interactions with immunosuppressive agents: Review of the in vitro functional assays and role of cytochrome P450 enzymes. *Transplantation*. 2008;**85**:1222-1229. DOI: 10.1097/TP.0b013e31816fc03b

- [133] Kaplan B, Friedman G, Jacobs M, Viscuso R, Lyman N, DeFranco P, Bonomini L, Mulgaonkar SP. Potential interaction of troglitazone and cyclosporine. *Transplantation*. 1998;**65**:1399-1400. DOI: 10.1097/00007890-199805270-00021
- [134] Oscarson M, Zanger UM, Rifki OF, Klein K, Eichelbaum M, Meyer UA. Transcriptional profiling of genes induced in the livers of patients treated with carbamazepine. *Clinical Pharmacology and Therapeutics*. 2006;**80**:440-456. DOI: 10.1016/j.clpt.2006.08.013
- [135] Cerveny L, Svecova L, Anzenbacherova E, Vrzal R, Staud F, Dvorak Z, Ulrichova J, Anzenbacher P, Pavek P. Valproic acid induces CYP3A4 and MDR1 gene expression by activation of constitutive androstane receptor and pregnane X receptor pathways. *Drug Metabolism and Disposition*. 2007;**35**:1032-1041. DOI: 10.1124/dmd.106.014456
- [136] Cooney GF, Mochon M, Kaiser B, Dunn SP, Goldsmith B. Effects of carbamazepine on cyclosporine metabolism in pediatric renal transplant recipients. *Pharmacotherapy*. 1995;**15**:353-356. DOI: 10.1002/j.1875-9114.1995.tb04373.x
- [137] Punyawudho B, Cloyd JC, Leppik IE, Ramsay RE, Marino SE, Pennell PB, White JR, Birnbaum AK. Characterization of the time course of carbamazepine deinduction by an enzyme turnover model. *Clinical Pharmacokinetics*. 2009;**48**:313-320. DOI: 10.2165/00003088-200948050-00003
- [138] Kashuba AD, Nafziger AN, Kearns GL, Leeder JS, Gotschall R, Rocci ML Jr, Kulawy RW, Beck DJ, Bertino JS Jr. Effect of fluvoxamine therapy on the activities of CYP1A2, CYP2D6, and CYP3A as determined by phenotyping. *Clinical Pharmacology and Therapeutics*. 1998;**64**:257-268. DOI: 10.1016/S0009-9236(98)90174-6
- [139] Jones DR, Ekins S, Li L, Hall SD. Computational approaches that predict metabolic intermediate complex formation with CYP3A4 (+b5). *Drug Metabolism and Disposition*. 2007;**35**:1466-1475. DOI: 10.1124/dmd.106.014613
- [140] Cummins D, Sekar M, Halil O, Banner N. Myelosuppression associated with azathioprine-allopurinol interaction after heart and lung transplantation. *Transplantation*. 1996;**61**:1661-1662. DOI: 10.1097/00007890-199606150-00023
- [141] Weiler S, Aellig N, Fauchère I, Jetter A, Corti N. Treatment of gout in a renal transplant patient leading to severe thrombocytopenia. *Journal of Clinical Pharmacy and Therapeutics*. 2014;**39**:571-572. DOI: 10.1111/jcpt.12190
- [142] Perez-Ruiz F, Gomez-Ullate P, Amenabar JJ, Zarraga S, Calabozo M, Herrero-Beites AM, Nolla JM. Long-term efficacy of hyperuricaemia treatment in renal transplant patients. *Nephrology, Dialysis, Transplantation*. 2003;**18**:603-606. DOI: 10.1093/ndt/18.3.603
- [143] Trück J, Laube GF, von Vigier RO, Goetschel P. Gout in pediatric renal transplant recipients. *Pediatric Nephrology*. 2010;**25**:2535-2538. DOI: 10.1007/s00467-010-1599-6
- [144] Stamp LK, Chapman PT. Gout and organ transplantation. *Current Rheumatology Reports*. 2012;**14**:165-172. DOI: 10.1007/s11926-012-0235-9

- [145] Colombo D, Lunardon L, Bellia G. Cyclosporine and herbal supplement interactions. *Journal of Toxicology*. 2014;**2014**:145325. DOI: 10.1155/2014/145325
- [146] Arayne MS, Sultana N, Bibi Z. Grape fruit juice-drug interactions. *Pakistan Journal of Pharmaceutical Sciences*. 2005;**18**:45-57
- [147] Nowack R. Review article: Cytochrome P450 enzyme, and transport protein mediated herb-drug interactions in renal transplant patients: Grapefruit juice, St John's Wort - and beyond. *Nephrology (Carlton, Vic.)*. 2008;**13**:337-347. DOI: 10.1111/j.1440-1797.2008.00940.x
- [148] Wanwimolruk S, Prachayasittikul V. Cytochrome P450 enzyme mediated herbal drug interactions (part 1). *EXCLI Journal*. 2014;**13**:347-391. DOI: 10.17877/DE290R-15628
- [149] Komoroski BJ, Zhang S, Cai H, Hutzler JM, Frye R, Tracy TS, Strom SC, Lehmann T, Ang CY, Cui YY, Venkataramanan R. Induction and inhibition of cytochromes P450 by the St. John's wort constituent hyperforin in human hepatocyte cultures. *Drug Metabolism and Disposition*. 2004;**32**:512-518. DOI: 10.1124/dmd.32.5.512
- [150] Gödtel-Armbrust U, Metzger A, Kroll U, Kelber O, Wojnowski L. Variability in PXR-mediated induction of CYP3A4 by commercial preparations and dry extracts of St. John's wort. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2007;**375**:377-382. DOI: 10.1007/s00210-007-0172-8
- [151] Barone GW, Gurley BJ, Ketel BL, Lightfoot ML, Abul-Ezz SR. Drug interaction between St. John's wort and cyclosporine. *The Annals of Pharmacotherapy*. 2000;**34**:1013-1016. DOI: 10.1345/aph.10088
- [152] Mai I, Krüger H, Budde K, Johne A, Brockmöller J, Neumayer HH, Roots I. Hazardous pharmacokinetic interaction of Saint John's wort (*Hypericum perforatum*) with the immunosuppressant cyclosporin. *International Journal of Clinical Pharmacology and Therapeutics*. 2000;**38**:500-502. DOI: 10.5414/CP38500
- [153] Ruschitzka F, Meier PJ, Turina M, Lüscher TF, Noll G. Acute heart transplant rejection due to Saint John's wort. *Lancet*. 2000;**355**:548-549. DOI: 10.1016/S0140-6736(99)05467-7
- [154] Hebert MF, Park JM, Chen YL, Akhtar S, Larson AM. Effects of St. John's wort (*Hypericum Perforatum*) on tacrolimus pharmacokinetics in healthy volunteers. *Journal of Clinical Pharmacology*. 2004;**44**:89-94. DOI: 10.1177/0091270003261078
- [155] Mai I, Bauer S, Perloff ES, Johne A, Uehleke B, Frank B, Budde K, Roots I. Hyperforin content determines the magnitude of the St John's wort-cyclosporine drug interaction. *Clinical Pharmacology and Therapeutics*. 2004;**76**:330-340. DOI: 10.1016/j.clpt.2004.07.004
- [156] Karliova M, Treichel U, Malago M, Frilling A, Gerken G, Broelsch CE. Interaction of *Hypericum perforatum* (St. John's wort) with cyclosporin a metabolism in a patient after liver transplantation. *Journal of Hepatology*. 2000;**33**:853-855. DOI: 10.1016/S0168-8278(00)80321-9
- [157] Ernst E. St John's Wort supplements endanger the success of organ transplantation. *Archives of Surgery*. 2002;**137**:316-319. DOI: 10.1001/archsurg.137.3.316

- [158] Alscher DM, Klotz U. Drug interaction of herbal tea containing St. John's wort with cyclosporine. *Transplant International*. 2003;**16**:543-544. DOI: 10.1007/s00147-003-0560-z
- [159] Mai I, Störmer E, Bauer S, Krüger H, Budde K, Roots I. Impact of St John's wort treatment on the pharmacokinetics of tacrolimus and mycophenolic acid in renal transplant patients. *Nephrology, Dialysis, Transplantation*. 2003;**18**:819-822. DOI: 10.1093/ndt/gfg002
- [160] Hermann M, Asberg A, Reubsaet JL, Sather S, Berg KJ, Christensen H. Intake of grapefruit juice alters the metabolic pattern of cyclosporin a in renal transplant recipients. *International Journal of Clinical Pharmacology and Therapeutics*. 2002;**40**:451-456. DOI: 10.5414/CP40451
- [161] Bailey DJ, Dresser G, Malcolm J, Arnold O. Grapefruit–medication interactions: Forbidden fruit or avoidable consequences? *Canadian Medical Association Journal*. 2013;**185**:309-316. DOI: 10.1503/cmaj.120951
- [162] Lin HL, Kent UM, Hollenberg PF. The grapefruit juice effect is not limited to cytochrome P450 (P450) 3A4: Evidence for bergamottin-dependent inactivation, heme destruction, and covalent binding to protein in P450s 2B6 and 3A5. *The Journal of Pharmacology and Experimental Therapeutics*. 2005;**313**:154-164. DOI: 10.1124/jpet.104.079608
- [163] Messer A, Raquet N, Lohr C, Schrenk D. Major furocoumarins in grapefruit juice II: Phototoxicity, photogenotoxicity, and inhibitory potency vs. cytochrome P450 3A4 activity. *Food and Chemical Toxicology*. 2012;**50**:756-760. DOI: 10.1016/j.fct.2011.11.023
- [164] Burkina V, Zlabek V, Halsne R, Ropstad E, Zamaratskaia G. In vitro effects of the citrus flavonoids diosmin, naringenin and naringin on the hepatic drug-metabolizing CYP3A enzyme in human, pig, mouse and fish. *Biochemical Pharmacology*. 2016;**110-111**:109-116. DOI: 10.1016/j.bcp.2016.04.011
- [165] Lin HL, Kenaan C, Hollenberg PF. Identification of the residue in human CYP3A4 that is covalently modified by bergamottin and the reactive intermediate that contributes to the grapefruit juice effect. *Drug Metabolism and Disposition*. 2012;**40**:998-1006. DOI: 10.1124/dmd.112.044560
- [166] Ho PC, Saville DJ, Wanwimolruk S. Inhibition of human CYP3A4 activity by grapefruit flavonoids, furanocoumarins and related compounds. *Journal of Pharmacy & Pharmaceutical Sciences*. 2001;**4**:217-227
- [167] Fujita T, Kawase A, Niwa T, Tomohiro N, Masuda M, Matsuda H, Iwaki M. Comparative evaluation of 12 immature citrus fruit extracts for the inhibition of cytochrome P450 isoform activities. *Biological & Pharmaceutical Bulletin*. 2008;**31**:925-930. DOI: 10.1248/bpb.31.925